Kidney failure related to broad-spectrum antibiotics in critically ill patients: secondary end point results from a 1200 patient randomized trial

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Complete List of Authors:	Jensen, Jens-Ulrik; Faculty of Health Sciences, University of Copenhagen, Copenhagen HIV Programme; University Hospital of Copenhagen, Bispebjerg, Pulmonary Medicine (L) Hein, Lars; Copenhagen University Hospital Hillerød, Department of Anesthesia and Intensive Care; Copenhagen University Hospital Glostrup, Department of Anesthesia and Intensive Care Lundgren, Bettina; University Hospital of Copenhagen Rigshospitalet, Diagnostic Centre Bestle, Morten; Copenhagen University Hospital Hillerød, Department of Anesthesia and Intensive Care Mohr, Thomas; Copenhagen University Hospital Gentofte, Department of Anesthesia and Intensive Care Andersen, Mads; Aarhus University Hospital in Skejby, Department of Anesthesia and Intensive Care Thornberg, Klaus; Copenhagen University Hospital Gentofte, Department of Anesthesia and Intensive Care Løken, Jesper; Copenhagen University Hospital Hvidovre, Department of Anesthesia and Intensive Care Steensen, Morten; Copenhagen University Hospital Hvidovre, Department of Anesthesia and Intensive Care Fox, Zoë; Royal Free Hospital School of Medicine in London, Research Department of Infection and Population Health; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Tousi, Hamid; Copenhagen University Hospital Herlev, Department of Anesthesia and Intensive Care Søe-Jensen, Peter; Copenhagen University Hospital Herlev, Department of Anesthesia and Intensive Care Lauritsen, Anne; Copenhagen University Hospital Glostrup, Department of Anesthesia and Intensive Care Strange, Ditte; Copenhagen University Hospital Glostrup, Department of Anesthesia and Intensive Care Reiter, Nanna; University Hospital in Roskilde, Department of Anesthesia and Intensive Care Thormar, Katrin; Copenhagen University Hospital Gentofte, Department of Anesthesia and Intensive Care Fjeldbord, Paul; Aarhus University Hospital in Skejby, Department of Anesthesia and Intensive Care Larsen, Kim; Aarhus University Hospital in Aarhus, Department of Anesthesia and Intensive Care Drenck, Niels-Erik; University Hospital in Roskilde, Department of

	Anesthesia and Intensive Care Østergaard, Christian; Copenhagen University Hospital Hvidovre, Clinical Microbiology Johansen, Maria; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Nielsen, Lene; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Kjær, Jesper; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Grarup, Jesper; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Lundgren, Jens; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme; Copenhagen University Hospital Rigshospitalet, Infectious Diseases
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Kidney failure related to broad-spectrum antibiotics in critically ill patients: secondary end point results from a 1200 patient randomized trial

Corresponding author Jens-Ulrik Jensen, Copenhagen HIV Programme, The Panum Institute, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen N, juj@cphiv.dk

Jens Ulrik Jensen *medical doctor*[1,2], Lars Hein *anaesthetist*[3,4], Bettina Lundgren *centre director, hospital diagnostic centre* [2,5], Morten Heiberg Bestle *anaesthetist* [4], Thomas Mohr *anaesthetist*[6], Mads Holmen Andersen *anaesthetist*[7], Klaus Julius Thornberg *anaesthetist*[6], Jesper Løken *anaesthetist*[8], Morten Steensen *anaesthetist*[8], Zoe Fox *biostatistician*[1,9], Hamid Tousi *anaesthetist*[10], Peter Søe-Jensen *anaesthetist*[10,] Anne Øberg Lauritsen *anaesthetist*[3], Ditte Gry Strange *anaesthetist*[3], Nanna Reiter *anaesthetist* [11], Katrin Thormar *anaesthetist*[6], Paul Christian Fjeldborg *anaesthetist* [7], Kim Michael Larsen *anaesthetist*[12], Niels-Erik Drenck *anaesthetist*[11] Maria Egede Johansen *junior research associate*[1], Lene Ryom *junior research executive*[1], Christian Østergaard *senior research executive*[2,13], Jesper Kjær *database manager*,[1] Jesper Grarup *administrative leader* [1], Jens D. Lundgren *professor of infectious diseases*,[1,14] of the The Procalcitonin And Survival Study (PASS) Group*.

[1]Copenhagen HIV Programme at the University of Copenhagen; [2]Department of Clinical Microbiology at Copenhagen University Hospital Hvidovre; [3]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Glostrup; [4]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Hillerød; [5]Diagnostic Centre at Copenhagen University Hospital Rigshospitalet; [6]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Gentofte; [7]Department of Anesthesia and Intensive Care at Aarhus University Hospital in Skejby; [8]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Hvidovre; [9]Royal Free Hospital School of Medicine in London; [10]Department of

Anesthesia and Intensive Care at Copenhagen University Hospital Herlev; [11]Department of Anesthesia and Intensive Care at Copenhagen University Hospital in Roskilde; [12]Department of Anesthesia and Intensive Care at Aarhus University Hospital in Aarhus; [13]Department of Clinical Microbiology at Copenhagen University Hospital Herlev; [14]Department of Infectious Diseases at Copenhagen University Hospital Rigshospitalet. All except[9] are from Denmark. [9] is from England.

*Participating investigators are listed in the appendix.

Running Title: Broad-Spectrum Antibiotics and Renal Failure in Critically Ill Patients

Keywords: Antibiotics – Renal Failure – Sepsis – Intensive Care

Summary:

Article Focus:

• To determine whether high exposure to beta-lactam, carbapenem and flour-quinolone antibiotics leads to renal failure in intensive care patients

• If renal failure is observed: To determine whether this renal failure is caused by antibiotics as a class of drugs or rather if one antibiotic or a certain combination leads to this renal failure.

Key messages:

• Patients randomised to 'high exposure' to antibiotics in the intensive care unit had substantially increased time with renal failure

• Patients who had piperacillin/tazobactam administered suffered the slowest rate of renal function recovery of all antibiotics tested.

• Adjustment for potential confounders did not change this signal and all sensitivity analyses also confirmed the findings. After discontinuation of piperacillin/tazobactam, renal function recovered with a rapid pace, indicating a reversible nephrotoxicity.

Strengths and Limitations:

• The randomised design, sample size of 1200 patients, Good Clinical Practice monitoring with high follow-up rates and broad eligibility criteria are powerful means of avoiding bias, confounding, and coincidental variation, and to assure a high external validity of the results.

• The study was, however, a secondary analysis. To compensate for this limitation, a detailed analysis plan was made before starting the study. Additionally, in the original protocol, analyses of renal function in the two arms was already planned.

Abstract

Objectives: To determine whether a strategy of more intensive antibiotic therapy with antibiotics not normally considered to be nephrotoxic leads to adverse renal outcomes in intensive care patients.

Design: Secondary analysis from a randomized antibiotic strategy trial (the *PASS study*). The randomized arms were conserved from the primary trial for the main analysis.

Setting: Nine mixed surgical/medical intensive care units across Denmark.

Participants: 1200 adult intensive care patients, 18 years or older, who were expected to stay more than 24 hours. Exclusion criteria were known extreme bilirubin >40 mg/dL or triglycerides >1000 mg/dL, patients at an increased risk from blood sampling, pregnant or breast feeding and persons held by force (psychiatric).

Interventions: Patients were randomized either to guideline-based therapy ('standard-exposure'-arm), or to guideline-based therapy supplemented with antibiotic escalation whenever procalcitonin increased ('high-exposure'-arm), according to daily measurements of this biomarker.

Main outcome measures: Renal failure, as defined by 1) RIFLE criteria, 2) estimated Glomerular Filtration Rate (eGFR) increase after administration of a certain drug, 3) eGFR) <60 ml/min/1.73 m^2 ('ever' or 'total time') until day 28. Analysis was by intention to treat.

Results: 28-day mortality was 31.8% and comparable (Jensen et al, CCM 2011). A total of 3672/7634 (48.1%) study days during follow-up in the "high-exposure" vs. 3016/6949 (43.4%) in the 'standard-exposure'-arm were spent with eGFR <60 ml/min/1.73m^2, p<0.001. In a multiple effects model, piperacillin/tazobactam was identified as causing the lowest rate of renal recovery of all antibiotics: 1.0 ml/min/m^2 per 24h while exposed to this drug [95% CI: 0.7 – 1.3 ml/min/m^2/24h] vs. meropenem: 2.9 ml/min/m^2/24h [2.5 – 3.3 ml/min/m^2/24h]); after discontinuing piperacillin/tazobactam, the renal recovery rate increased: 2.7 ml/min/1.73 m^2 /24h [2.3 – 3.1

ml/min/1.73 m^2 /24h]). eGFR<60 ml/min/1.73m^2 in the two groups at entry and at last day of follow-up was 57% vs. 55% and 41% vs. 39%, resp.

Conclusions: Piperacillin/tazobactam was identified as a cause of delayed renal recovery in critically ill patients. This nephrotoxicity was not observed when using other beta-lactam antibiotics. It remains unclear, whether such a nephrotoxic effect is also present in non-critically ill patients.

Trial registration ClinicalTrials.gov identifier NCT00271752.

Introduction

Frequent complications to sepsis are organ failure, especially respiratory failure and renal failure [1-3]. Critically ill patients are more vulnerable to organ-related drug toxicities than less severely ill patients[4]. Randomized trials assessing safety of broad-spectrum antibiotics in intensive care settings are generally scarce, do not have sufficient statistical power for assessing organ failure endpoints, and do often not include defined kidney organ failure endpoints[5-7]. Data on renal failure endpoints are also sparse in the published trials from other patient populations, and since the absolute risk of renal failure is low for these patients, analyses may likely have been underpowered[8-12].

To our knowledge, randomized trials comparing 'high exposure' vs. 'standard exposure to antibiotics' and specifically addressing whether these interventions affect the occurrence and duration of kidney failure have not been done before in intensive care settings.

In this secondary analysis from a randomized trial, the PASS study[13], we aimed to investigate whether a strategy of more intensive antibiotic therapy leads to adverse renal outcomes within 28 days after recruitment.

Secondly, if renal failure was observed from the 'high exposure' approach, to identify one or several of the antibiotics used in this trial as the cause of such a renal failure.

Methods

Trial design and participants

PASS is a multicentre randomized controlled trial in Denmark 2006-9 in 1200 adult critically ill patients, expected to stay in one of the nine participating mixed medical/surgical intensive care units ≥24 hours; the CONSORT trial diagram is displayed in figure 1. Patients were randomized 1:1 either to treatment according to international guidelines: 'standard exposure arm', or to same guidelines but supplemented with daily drug-escalation initiated upon procalcitonin increases ('high exposure'-arm); 28-day mortality was 31.8% and comparable between the two groups, as reported[13].

To be eligible, patients had to be ≥18 years, enrolled within 24 hours of admission to the intensive care unit and have an expected intensive care-admission length of ≥ 24 hours. Patients with known bilirubin >40 mg/dL and triglycerides >1000 mg/dL (not suspensive) were not eligible (interference with procalcitonin measurements), as were patients who were judged to be at an increased risk from blood sampling. The inclusion criteria were broad since infection is frequent and often causes complications in the patient group and to increase the external validity of the results. The person or next of kin gave informed consent. The study protocol was approved by the regional ethics committees in Denmark (H-KF-272-753) and adheres to the Helsinki declaration, revised in Seoul 2008.

In the present analyses we explored presence and duration of renal failure as well as change in renal function during the observed time. Endpoints are defined in *statistical analysis* below. Patients were followed until day 28. The primary trial protocol and the analysis plan is available in the online supplement. Analysis was by intention to treat: NCT00271752.

Randomization and masking

Randomization was performed 1:1 using a computerized algorithm created by the database manager (JK) with concealed block-size, pre-stratified for site of recruitment, initial APACHE-II and age

(entered in an encrypted screening form in a password protected website); investigators were masked to assignment before, but not after, randomization. All investigators were trained by the coordinating centre and had to register in an investigator-database. Investigators, treating physicians and the coordinator were unaware of outcomes during the study, as were they of all procalcitonin measurements in the 'standard exposure' (control)-group.

Antibiotic therapy in the two arms

The investigators enrolled participants and assigned the 'high exposure group' participants to the intervention. In the 'standard exposure' group, the antimicrobial treatment was guided according to current clinical guidelines[14], based on clinical assessment, microbiology and radiology among other parameters, as described elsewhere[13]

In the 'high exposure' group, the use of antimicrobial interventions was guided by the same clinical guidelines as in the 'standard exposure' group to ascertain the best standard of care therapy for all patients, and additionally antimicrobial interventions were initiated whenever procalcitonin levels were not decreasing at a pre-defined pace (figure 2) and diagram D1 in the online supplement where a site-adjusted local guideline is displayed.

Measurements, data collection and follow-up

Blood samples for biomarker measurement were made daily in the intensive care unit, beginning immediately after randomization. The assay used was the Kryptor®-PCT. Organ failure and antibiotic exposure was followed up for until 28 days or death, as described[13]. Good Clinical Practice guidelines were applied. The regional ethics board approved the protocol (H-KF-01-272-753).

Statistical analysis

Analyses for renal failure endpoints were divided into: I) dichotomous endpoints to explore whether renal failure emerged during therapy with the investigated antibiotics and II) quantitative endpoints to explore whether existing renal failure was prolonged during therapy. Dichotomous endpoints were: 1) RIFLE-criteria 'R', 'I' and 'F' www.adqi.net, 2) 'ever' eGFR<30 or 60 ml/min/1.73m^2, 3) 'ever' blood-urea level ≥20 mmol/L. Quantitative endpoints were based on the time lived with eGFR<30 or 60 ml/min/1.73m^2 and the day-to-day change in eGFR.

The multiple effects eGFR 'slope' analyses, were adjusted for the following variables: treatment arm ('high exposure' vs. 'standard exposure'), age (≥65 vs. <65 years), gender, baseline APACHE II score (≥20 vs. <20), degree of host response/infection at baseline (severe sepsis/septic shock vs. milder or no infection as defined[15]), the eGFR at initiation of the investigated antibiotic, and finally, whether the patient at baseline was considered to be 'surgical' or 'medical'.

Comparisons were made between treatment arms using Students t-tests (for normal distributed continuous data) and Mann-Whitney U-tests (for non-normally distributed continuous data). Chi-squared tests and logistic regression models were used to test categorical variables. Time-to-event analyses comparing the 'high exposure' group with the 'standard exposure' group were performed using Kaplan-Meier plots and Cox proportional hazards models. Interactions were explored whenever an interaction could be rationally expected according to background literature, for the multivariate models performed. Statistical analyses were performed using STATA Version 10.2, and SAS version 9.1. All reported p-values are 2-sided using a level of significance of 0.05.

Sample size

For the present hypothesis, two sample size calculations were performed; one for a chi-square for equal proportions analysis for the originally randomized arms, and one for a multivariable logistic regression analysis, both with a limit for type I error of 5% and a power to avoid type II error of

80%. For the chi-square analysis, using a premise of the endpoint occurring in 20% of patients in the 'standard exposure' group and with 1200 patients randomized, a detection limit (one-sided) for relative risk of 1.3 in the 'high exposure' group was established. For the multivariate approach, the summed squared correlations (Σrho^2) to the risk of the antibiotic drug investigated, was set to 0.3. The frequency of the endpoint in the 'standard exposure' group and the sample size were set as for the chi-square analysis and the frequency of the exposure was set at 30%, which resulted in a detection limit for odds ratio of ≥1.5 (or ≤0.67).

Results

Baseline characteristics

Nine sites included 1200 persons between 09/01/06 and 02/06/09. Eighty-three percent of the patients were assessed by the investigator to have an infection at baseline and 81% of the patients suffered from chronic co-morbidity. Table 1 briefly summarizes baseline characteristics. Mortality was comparable between the two groups, as reported[13].

Follow-up

Follow-up for renal measures during the 28-day study period was made on 9,348 days in the 'standard-exposure' group of 10,755 days alive and admitted to hospital (86.9%) vs. 9,866 of 11,380 days in the 'high exposure group' (86.7%). If time after discharge from hospital (where no S-creatinine values were determined) until day 28 was included, the percentage of days with assessment of renal failure was 71.2% (9,348/13,130 days) vs. 73.8% (9,866/13,377 days)."

Use of Antibiotics

The antibiotics used most while admitted to the ICU were piperacillin/tazobactam, cefuroxim, meropenem and ciprofloxacin, and there was a substantial higher use of piperacillin/tazobactam and

ciprofloxacin in the 'high exposure' arm (table 2). Vancomycin was used to a lesser extent in both groups and aminoglycosides and colistin were used rarely in both groups.

The median length of an antibiotic course was prolonged using the 'high exposure'-algorithm (6 days (IQR 3, 11) vs. 4 days (IQR 3, 10), p=0.004.

Renal failure in the originally randomized study arms

The % of days within day 1-28 with eGFR≤ 60 ml/min/m^2 was 48% in the 'high exposure' arm vs. 43% in the 'standard exposure' arm, p<0.0001. Results in table 3 are estimated eGFR values, based on actual measured S-creatinine values; results regarding days with eGFR were comparable if using the 'last observation carried forward' approach (not shown). RIFLE-criterion 'R' occurred more often within day 1-28 in the 'high exposure' arm than the 'standard exposure' arm: 209 patients vs. 170 patients, p=0.02, as did blood urea levels exceeding 20 mmol/L: 253 (43.4%) vs. 217 (37.4%), p=0.04.

The frequency of renal failure on the last day of follow-up was comparable between the arms (table 4), underlining that the results depicted in table 3 reflect a temporary extension of duration of renal failure in the "high exposure group" and furthermore that this observation is not explained by premature discharge of renally incompetent patients in the 'standard exposure' arm.

Glomerular Filtration Rate changes and exposure to certain antibiotics

Comparison of the eGFR of all patients (both study arms) for the first ten days after starting on the most frequently used betalactam antibiotics showed that the slowest recovery of renal function was observed in patients on piperacillin/tazobactam as compared to patients on meropenem or cefuroxim (figure 3). A multiple effects model investigating the eGFR regression coefficient ('increase in eGFR') per day on these drugs confirmed that renal recovery was lowest in patients on piperacillin/tazobactam (figure 4). Of note, renal recovery seems to be low in patients exposed to

cefuroxim, but as displayed in fig. 3, this drug is given to patients with a relatively normal renal function (leaving few possibilities for 'recovery').

For the first five days following discontinuation of these drugs, adjusting for the same variables, eGFR increased: piperacillin/tazobactam, 2.7 ml/min/1.73 m^2 [95% CI: 2.3 – 3.1 ml/min/1.73 m^2]); meropenem, 0.2 ml/min/1.73 m^2 [-0.5 – 0.9], cefuroxim, 0.0 ml/min/1.73 m^2 [-0.4 – 0.4].

As a sensitivity analysis a logistic regression model with forward censoring of variables was built, where the endpoint was 'eGFR<60 ml/min/1.73 m^2 at day seven from study entry'. Variables were included if they were associated with the endpoint with p<0.1). Patients who died or who were discharged from hospital before day seven were counted with their last eGFR measurement. Use of piperacillin/tazobactam for at least three days within these first seven days was found to be an independent predictor of eGFR<60 ml/min/1,73 m^2 at day seven (OR: 1.6 [95% CI: 1.1 – 2.4]), whereas treatment with cefuroxim (OR: 1.2 [95% CI: 0.8 – 1.8]) or meropenem (OR: 0.9 [95% CI: 0.5 – 1.4]) for three days or more were not predictors of this endpoint. The following modifications did not alter the signal of this analysis: 1) excluding all patients who died within the first seven days, 2) excluding all patients with invasive fungal infection on day 1-28, 3) combining the betalactam exposure with exposure to flour-quinolone exposure (data not shown) or 4) adding 'Alert-procalcitonin' at baseline as a variable.

Discussion

Principal findings

We observed that the duration of renal failure is prolonged in critically ill patients randomized to receive high exposure to broad-spectrum antibiotics and escalated diagnostic work-up according to a biomarker-strategy, compared to patients randomized to receive standard care according to guidelines regarding use of antibiotics and diagnostics. This difference in renal function was mainly

confined to a prolongation of existing renal dysfunction, since there was only a moderate, although significant, difference in de novo acute renal failure.

To our knowledge, this study provides the first substantive evidence to inform this critical issue within ICU medicine. Firstly, the study was a randomized, good clinical practice controlled trial with a high sample size for comparison of organ failure, and the patients' baseline characteristics in general and specifically regarding renal parameters, were comparable. Secondly, the rate of follow-up, although not complete for the entire period, was high and equal among the groups and the rate of renal failure on the last day of follow-up in the two groups was comparable. Thus, the observed increased risk of persistent renal failure in the "high-exposure group" is attributable to this intervention in some way.

The intervention consisted of an increased number of culture samples, a proposed initiative to do further diagnostic imaging (no observed difference) and a rapid and aggressive antibiotic escalation with certain drugs, which was documented to be of substantial extent (table 2). As a moderate increase in microbiologic sampling would not cause renal failure, and since there was no observed increase in diagnostic imaging, these interventions seems implausible reasons to explain the observations depicted in table 3.

This leaves us with the documented (table 2) escalation in use of piperacillin/tazobactam and ciprofloxacin as possible explanations. Before concluding, that the observed renal dysfunction was caused directly by one (or both) of these drugs, we wanted to exclude the possibility that the results had appeared because of a derived effect of an increase in fungal infections. Fungal infections have been linked to broad-spectrum antibiotics[16], and renal failure is a well-known complication to some antifungals[17]. However, excluding all patients with invasive fungal infections did not alter the results.

Based on these results, and after having excluded other potential explanations, we realized

that nephrotoxicity from piperacillin/tazobactam and/or ciprofloxacin was the most plausible explanation of the observed renal dysfunction. To further substantiate this, several analyses were conducted. A multiple effects model was built to examine the GFR in the days after administration of different frequently used drugs. This model included the five most often administered antibiotics, including piperacillin/tazobactam, meropenem, cefuroxim, ciprofloxacin and vancomycin along with other known and suspected causes of renal failure. In this model, the use of piperacillin/tazobactam was associated with a striking low rate of GFR-improvement, compared to the other drugs investigated. Intriguingly, this adverse effect appears to be reversible, since patients in whom, piperacillin/tazobactam was discontinued, had the fastest improvement in renal function as compared with patients on other antibiotic courses. Several sensitivity analyses were performed with findings consistent with this observation.

Comparison with other studies

Although clinical evidence regarding renal failure according to use of piperacillin/tazobactam in ICU patients has been limited, the influence of piperacillin on renal function has been investigated in healthy volunteers in laboratory experiments. In a cross-over experiment, the influence on drug clearance from concurrent administration of piperacillin and flucloxacillin was estimated[18]. The authors observed that flucloxacillin clearance was reduced to 45% [90% CI: 40 – 50%] when piperacillin was administered simultaneously, whereas piperacillin clearance was unaffected by concurrent flucloxacillin administration. Time-clearance slope modeling identified competitive inhibition of renal tubular secretion as the most likely explanation. Piperacillin-induced reduction of imipenem clearance[19] and of tazobactam clearance has also been found[20], and a high correlation between creatinin clearance and piperacillin clearance has been documented[21], and thus, it is plausible that piperacillin specifically causes nephrotoxicity.

Additionally, the published randomized trials comparing piperacillin/tazobactam with other beta-lactam drugs in intensive care unit settings are scarce, underpowered for assessment of renal failure endpoints and do generally not address renal endpoints[5-7]. Trials from other settings: haematological patients, diabetes patients, and surgical settings do generally not investigate renal failure endpoints, and in the few (non-ICU) trials that do report kidney endpoints, the total frequency of these makes the power to avoid type II error very low (diagram D2, online digital supplement).

Strengths and weaknesses of the study

Although our study is performed on analyses from a large randomized good clinical practice controlled trial with a stringent methodology and a high level of follow-up, there are limitations that deserve mentioning: First, follow-up for organ-related measures was not complete, although we followed patients for all blood samples done in 1) the hospital, at which they were initially recruited, 2) other hospitals in Denmark, where we had electronic access to blood samples. However, patients who continued to suffer from renal failure when discharged from hospital, were out of reach for follow-up for their renal function. Of note, the fraction of patients with remaining renal failure at time of discharge was comparable between the two groups (table 4), and hence it is unlikely that this lack of ability to ascertain renal outcome contributed to our main findings. Second, the study was a post hoc analysis using a previously published trial as material. We have tried to compensate for this by writing a detailed analysis-plan based on the hypotheses, we wanted to test, before analysis. Third, although the sample size was relatively large compared to most other randomized trials in this setting, the sample size for these secondary analyses were based on the assumption of 25% renal failure in the 'standard exposure group' and a relative risk of 1.25 in the 'high exposure group'. The observed numbers were 21% and 1.22 which calls for a slightly higher sample size. However, the sample size needed to show the differences observed in the multivariable

analyses was far smaller, and since these analyses confirmed the main findings, we do not think the results are due to chance.

In this trial, for the first time ever to our knowledge, random allocation to high exposure to broad-spectrum antibiotics in the intensive care unit has been systematically applied according to a randomized algorithm and this resulted in prolongation of renal failure. The results were confirmed when excluding patients with fungal infections, and a multiple effects model revealed a particularly low renal recovery in patients while piperacillin/tazobactam was administered and a remarkable recovery when discontinuing this drug; a finding that was specific for this drug. Several other crude and adjusted models likewise confirmed the findings. Finally, the results from this trial are supported by human experimental studies.

Conclusion

In conclusion, the use of piperacillin/tazobactam caused a delayed renal recovery in critically ill patients, and renal function improved after discontinuation of the drug. We cannot within the sample size of this trial establish whether the use of piperacillin/tazobactam in some cases causes persistent renal failure, and thus, further research to explore this is warranted. We think this impact on renal function is more likely caused by a toxic effect on the renal tubule than by a lack of effect towards the infection, since this drug is independently associated with a high chance of survival in other infected populations[8], and we must emphasize that our findings are strictly confined to critically ill patients.

Contributors

JUJ designed the study, made the data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, and drafted and the paper. He is guarantor. JUJ, ZF and JK cleaned and analysed the data. JL, BL, LH, MHB, TM, MHA, KJT, JL, MS, HT, PS-J, AØL, DGS,

NR, KT, PCF, KML, NED, MEJ, LR, CØ, ZF, JK and JG made input study design, data collection tools and analysis plan and on the manuscript. JUJ implemented the trial at the centers. All members of the Procalcitonin And Survival Study (PASS) Group assisted in designing the trial. The members of the PASS study group are as follows: Central Coordinating Centre - J.U. Jensen, B. Lundgren, J. Grarup, M.L. Jakobsen, S. S. Reilev, M. Kofoed-Djursner, J. D. Lundgren; Regional Coordinating Centres - Hvidovre - J. Løken, M. Steensen; Gentofte - T. Mohr, K. Thornberg, K. Thormar; Hillerød - L.Hein, M. Bestle; Glostrup - D. Strange, A.Ø. Lauritsen; Herlev - H. Tousi, P. Søe-Jensen; Roskilde - N. Reiter, N.E. Drenck; Skejby - M.H. Andersen, P. Fjeldborg; Århus - K.M. Larsen; Data Management & Statistical Centre - Z. Fox, J. Kjær, D. Kristensen; Procalcitonin Analysis & Logistics Centre - J.U.Jensen, B. Lundgren, M. B. Rasmussen, C. S.v.Hallas, M. Zacho, J. Iversen, T. Leerbeck, M. Jeppesen, K.S. Hansen, K.B. Jensen; Data and Safety Monitoring Board - H. Masur (Chair), J. Chastre, H. Schønheyder, C. Pedersen; Clinical Microbiology Management – B. Lundgren, J. D. Knudsen, A. Friis-Møller, K. Schønning, A. Lester, H. Westh, G. Lisby, J.K. Møller, B. Bruun, J.J. Christensen, C. Østergaard, M. Arpi, K. Astvad, M.D. Bartels, J. Engberg, H. Fjeldsøe-Nielsen, U.S. Jensen; PASS Site Clinical Investigators (numbers of recruited persons are in parentheses) - Glostrup (290) – L. Hein, T. Mohr, D. G. Strange, P. L. Petersen, A. Ø. Lauritsen, S. Hougaard, T. Mantoni, L. Nebrich, A. Bendtsen, L.H. Andersen, F. Bærentzen, Andreas Eversbusch, B. Bømler, R. Martusevicius, T. Nielsen. P.M. Bådstøløkken, C. Maschmann, U. Grevstad, P. Hallas, A. Lindhardt, T. Galle, K. Graeser, E. Hohwu-Christensen, P. Gregersen, H.C. Boesen, L.M. Pedersen, K. Thiesen, L.C. Hallengreen, I. Rye, J. Cordtz, K.R. Madsen, P.R.C. Kirkegaard, L. Findsen, L.H. Nielsen, D.H. Pedersen, J.H. Andersen, C. Albrechtsen, A. Jacobsen, T. Jansen, A.G. Jensen, H.H. Jørgensen, M. Vazin; Gentofte (209) – L. Lipsius, K. Thornberg, J. Nielsen, K. Thormar, M. Skielboe, B. Thage, C. Thoft, M. Uldbjerg, E. Anderlo, M. Engsig, F. Hani, R.B. Jacobsen. L. Mulla, U. Skram; Herlev (154) – H. Tousi, P. Søe-Jensen, T. Waldau, T. Faber, B. Andersen, I. Gillesberg, A. Christensen,

C. Hartmann, R. Albret, D.S. Dinesen, K. Gani, M. Ibsen; Hvidovre (148) – J. Løken, M. Steensen, J.A. Petersen, P. Carl, E. Gade, D. Solevad, C. Heiring, M. Jørgensen, K. Ekelund, A. Afshari, N. Hammer, M. Bitsch, J.S. Hansen, C. Wamberg, T.D. Clausen, R. Winkel, J. Huusom, D.L. Buck, U. Grevstad, E. Aasvang, K. Lenz, P. Mellado, H. Karacan, J. Hidestål, J. Høgagard, J. Højbjerg, J. Højlund, M. Johansen, S. Strande; Hillerød (138) – M. Bestle, S. Hestad, M. Østergaard, N. Wesche, S.A. Nielsen, H. Christensen, H. Blom, C.H. Jensen K. Nielsen, N.G. Holler, K.A. Jeppesen; Århus-Skejby (94) – M.H. Andersen, P. Fjeldborg, A. Vestergaard, O. Viborg, C.D. Rossau; Roskilde (90) – N. Reiter, M. Glæemose, M.B.Wranér, C.B. Thomsen, B. Rasmussen, C. Lund-Rasmussen, B. Bech, K. Bjerregaard, L. Spliid, L.L.W. Nielsen, N.E. Drenck; Århus-Centre (63) – K.M. Larsen, M. Goldinger, D. Illum, C. Jessen, A. Christiansen, A. Berg, T. Elkmann, J.A.K. Pedersen, M. Simonsen; Bispebjerg (14) H. Joensen, H. Alstrøm, C. Svane, A. Engquist.

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Supported by grants from the Danish Research Council, The Lundbeck Foundation, Research Foundation for the Capital Region of Denmark, The Toyota Foundation, Brahms diagnostica (un-restricted grant), The Harboe Foundation, The A.P. Møller Foundation and the Idella Foundation. None of these had any influence on the design or conduct of the study; collection, management, analysis, and interpretation of the data; nor the preparation, or approval of the manuscript.

Competing interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that the trial was funded mainly by the Danish State (Danish Research Council) and : all authors state that they have no relationships with companies that might have an interest in the submitted work in the previous 3 years; their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and all authors have no non-financial interests that may be relevant to the submitted work.

Ethical approval

The study was approved by the ethics committee for Copenhagen and Frederiksberg community (now Ethics Committee for the Capitol Region): H-KF-01-272-753. Patient consent: We received written consent from the patient or the next of kin for trial inclusion.

Data sharing

No additional data available.

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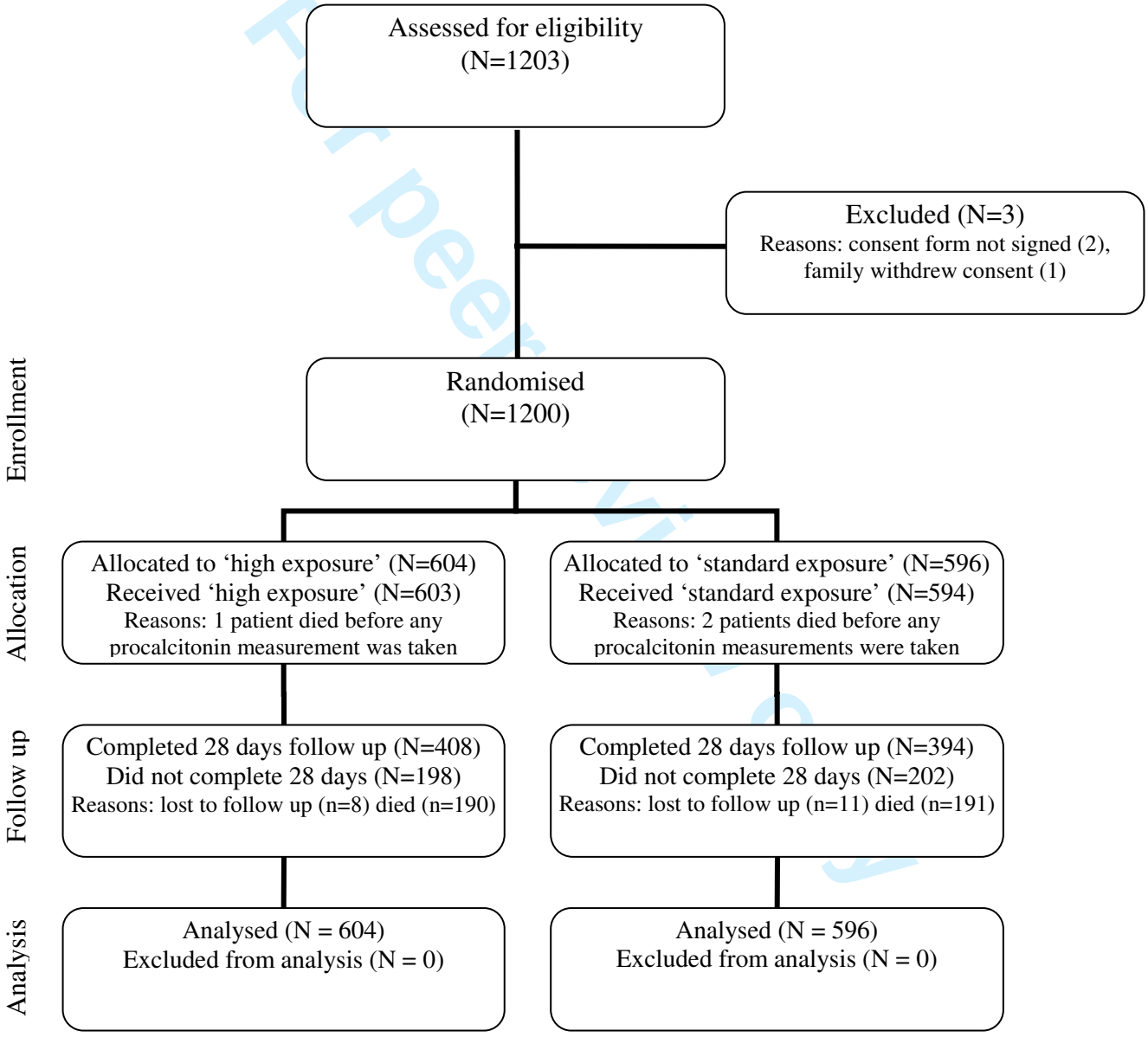


Figure 1. Patient Flow Diagram of the trial

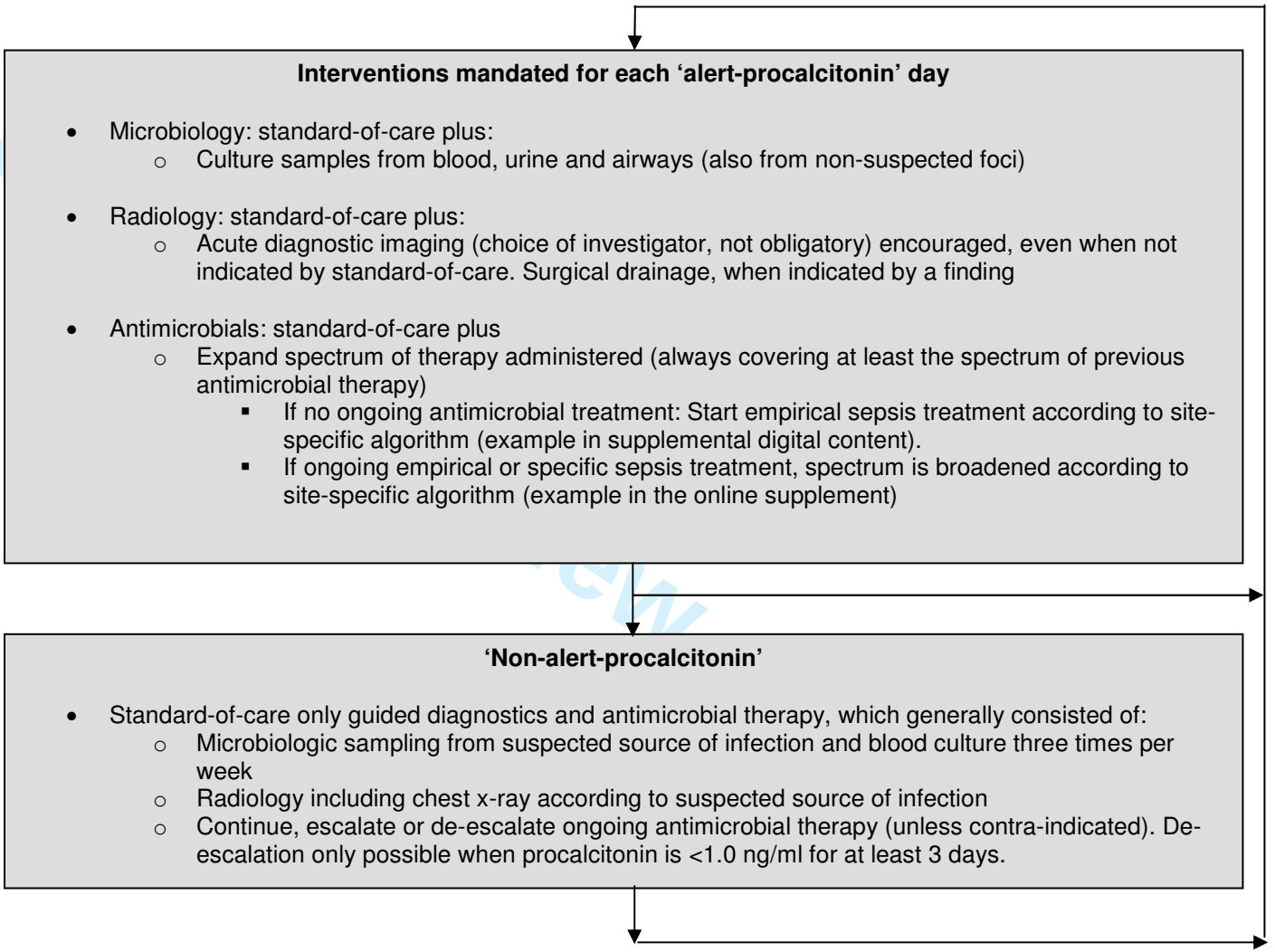

Interventions mandated for each 'alert-procalcitonin' day
Microbiology: standard-of-care plus:
Culture samples from blood, urine and airways (also from non-suspected foci)
Radiology: standard-of-care plus:
Acute diagnostic imaging (choice of investigator, not obligatory) encouraged, even when not indicated by standard-of-care. Surgical drainage, when indicated by a finding
Antimicrobials: standard-of-care plus
Expand spectrum of therapy administered (always covering at least the spectrum of previous antimicrobial therapy)
If no ongoing antimicrobial treatment: Start empirical sepsis treatment according to site-specific algorithm (example in supplemental digital content).
If ongoing empirical or specific sepsis treatment, spectrum is broadened according to site-specific algorithm (example in the online supplement)
'Non-alert-procalcitonin'
Standard-of-care only guided diagnostics and antimicrobial therapy, which generally consisted of:
Microbiologic sampling from suspected source of infection and blood culture three times per week
Radiology including chest x-ray according to suspected source of infection
Continue, escalate or de-escalate ongoing antimicrobial therapy (unless contra-indicated). De-escalation only possible when procalcitonin is <1.0 ng/ml for at least 3 days.

Figure 2. General principles of procalcitonin-guided intervention.

At 'alert-procalcitonin' situation (≥ 1.0 ng/ml and not decreasing by at least 10% from the previous day), interventions were obligatorily conducted according to an algorithm with specific instructions for intervention, which was adapted to the antimicrobial guidelines on the site. Antimicrobials were daily adjusted according to 1) present and previous procalcitonin values, 2) infectious state of the patient (clinical presentation, microbiology, radiology etc.) and 3) history of antimicrobial use. Procalcitonin-guided antimicrobial escalation was mandatory, except when 1) there was a clear contra-indication for administering it or 2) microbiology "explaining the infectious presentation of the patient" was announced (same date) leading to specific therapy. Standard-of-Care antimicrobial diagnostics and treatment was not waived in the 'high exposure arm (nor the 'standard exposure'arm) to assure patient safety. According to the standard-of-care principle, all patients with septic shock were treated at the onset of hypotension with antimicrobials covering >95% of the causes of this condition in our hospitals. Awaiting procalcitonin results/low procalcitonin levels was not considered a plausible reason to withhold antimicrobial treatment. The treating physician was reminded daily via phone from the coordinating centre at each 'alert-procalcitonin' to intervene. In the 'standard exposure' arm, procalcitonin measurements were not available.

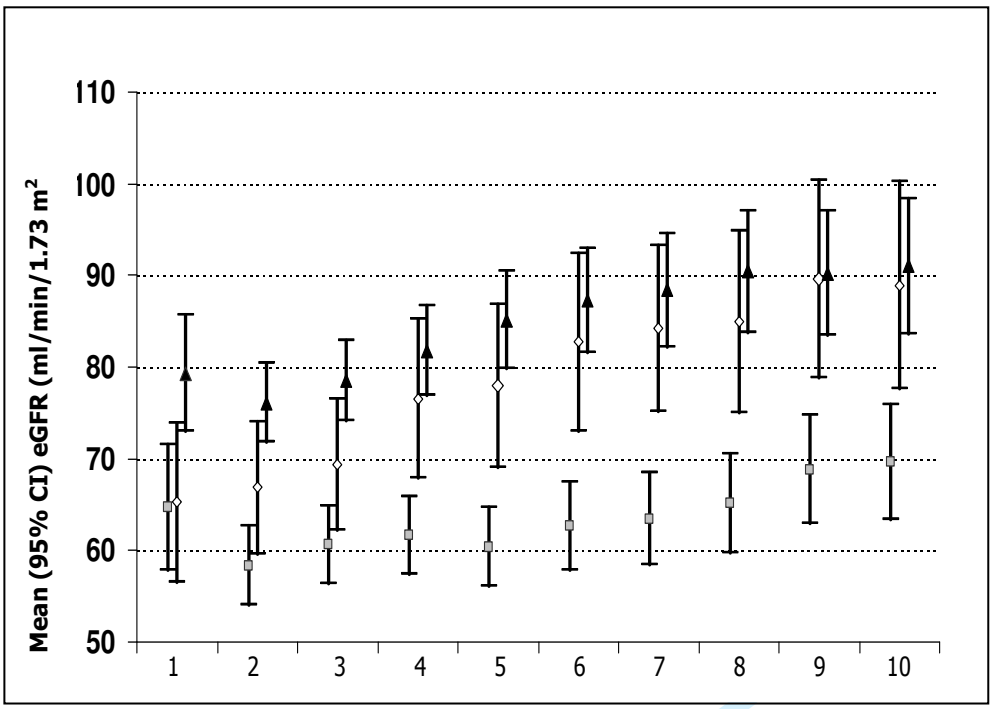


Figure 3. eGFR during ten days on cefuroxim, piperacillin/tazobactam and meropenem. ▲=cefuroxim; ▢=piperacillin/tazobactam; ◇=meropenem.

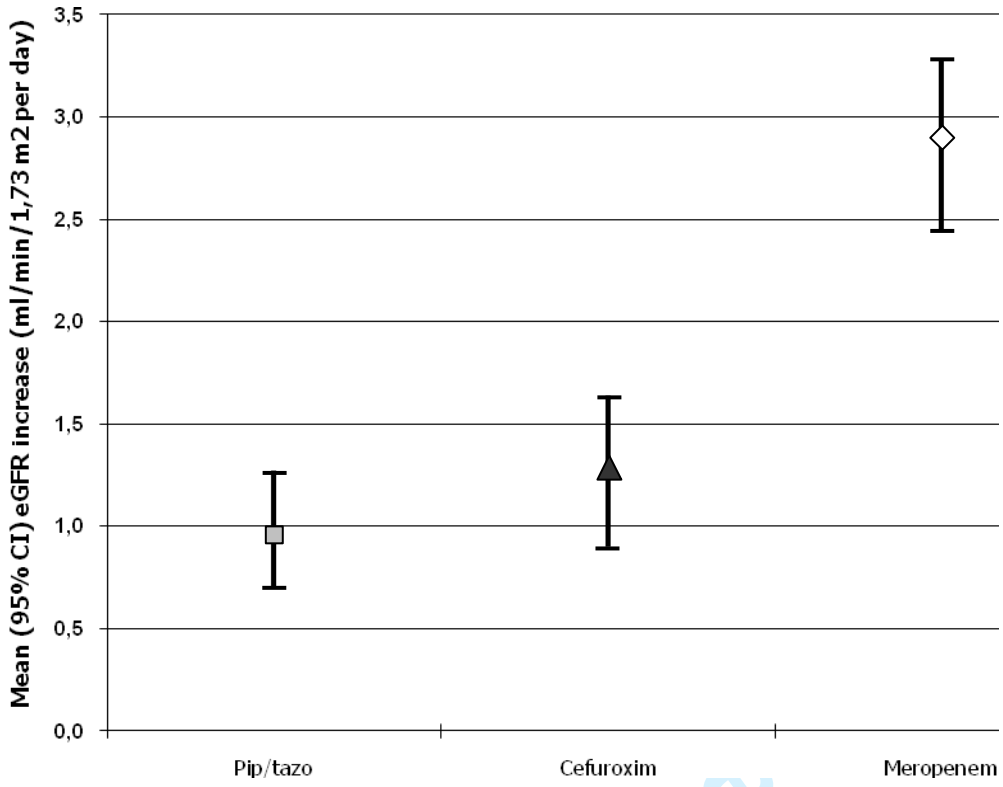


Figure 4. eGFR increase estimated per day use of antibiotics. Estimates were made for every antibiotic in mixed effect models, and all eGFR estimates were adjusted for: treatment arm ('low exposure' vs. 'high exposure'), gender, age (≥65 vs. <65 years), APACHE II score (≥20 vs. <20), Clinically judged infection (severe sepsis/septic shock vs. milder or no infection), patient category (surgical vs. medical) and eGFR level at administration of the antibiotic, (1: <30 ml/min/1,73 m^2, 2: 31-60 ml/min/1,73 m^2, 3: >60 ml/min/1,73 m^2). Pip/tazo=piperacillin/tazobactam).

Diagram D1 Example of the site-specific interventional algorithm, site 'Aarhus'

The Procalcitonin And Survival Study (PASS) Intervention Algorithm, Site: Aarhus

IMPORTANT: All patients shall (at least) receive antimicrobial therapy covering "standard-of-care", i.e. if any existing guidelines or evidence for antimicrobial treatment indicate/ contra-indicate surgical and/or antibiotic treatment, then the patient should be treated according to this. Indicated treatment should never be left out because of a possibly low procalcitonin (PCT).

All (except for the above standing situations) patients in the "PCT intervention" group must have treatment according to the present guidelines, including interventions when procalcitonin is ≥1,0 ng/ml and "Alert"[a].

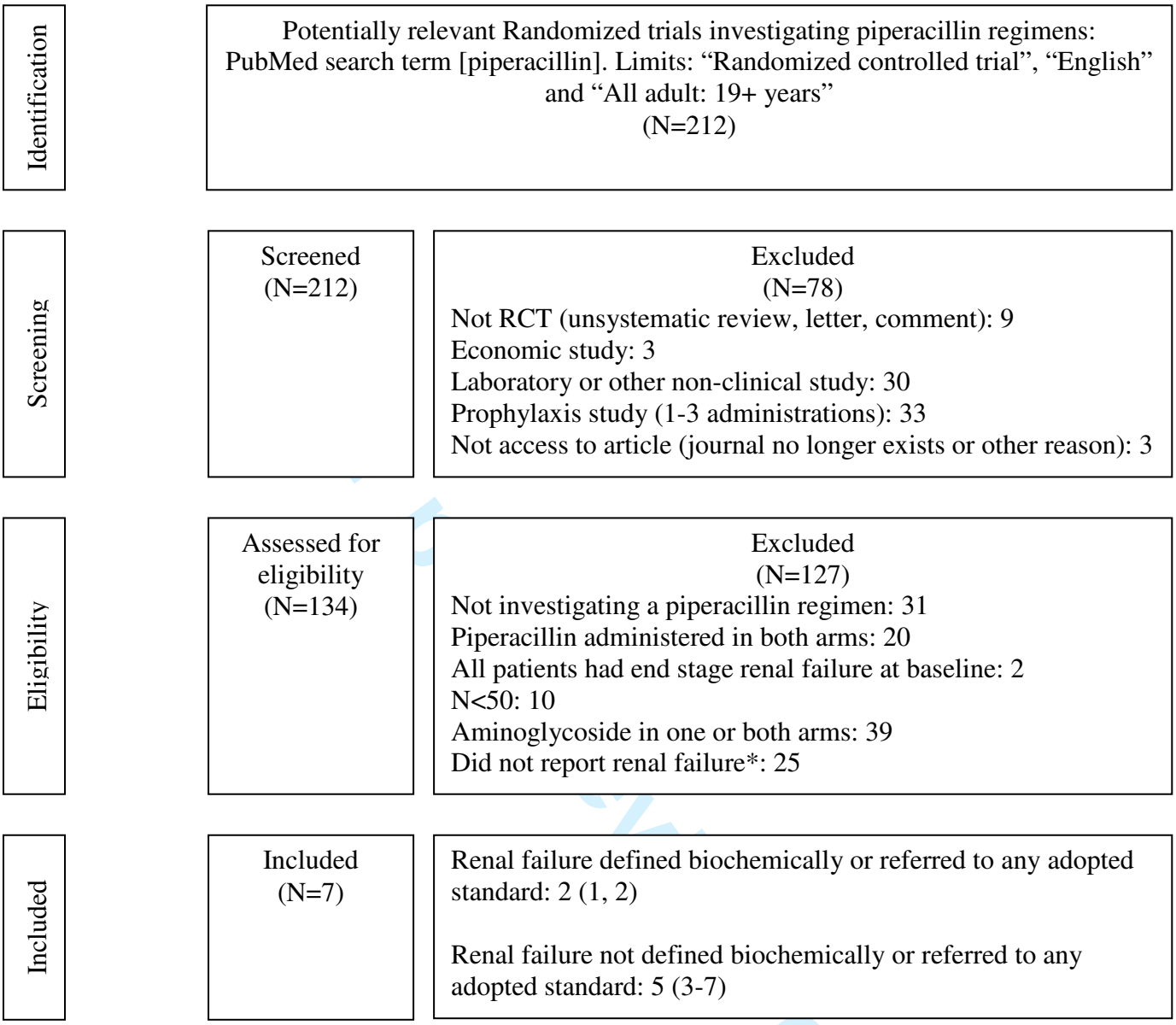
Patients are categorized daily according to the PASS intervention categories, on the basis on the present and the previous PCT measurement (displayed as "Alert" or "Non-Alert" in the website). In correspondence with every category, a PASS-intervention is displayed below. The treatment is, adjusted according to new and relevant microbiology that "explains" the clinical picture

CATEGORY 1	First PCT > 1,0 ng/ml, patient has not received antibiotics (≥1 DDD[b] within 72 h)
CATEGORY 2	A) First PCT ≥1,0 ng/ml, patient has received antibiotics (≥ DDD[b] within 72 h) or B) PCT "Alert" for 1 day after CAT 1,CAT 4 or CAT 5 has been started or C) PCT "Alert"** from "start-sample" till next morning
CATEGORY 3	A) First PCT ≥1,0 ng/ml, patient has received antibiotics (≥ DDD[b] within 72 h) and clinical suspicion of fungal infection or catheter related infection. or B) PCT "Alert" for 1 day after CAT 2 has been started
CATEGORY 4	A) Start PCT< 1,0 ng/ml or B) "Non-Alert" PCT, but ≥ 1,0 ng/ml. or C) PCT < 1,0 for 1-2 days
CATEGORY 5	PCT < 1,0 ng/ml for 3 or more days.

Action / Category	Diagnostics	Surgery	Antimicrobials[c]
CATEGORY 1	• Blood culture • Tracheal secretion • Urine culture • Culture from susp. source • Diagnostic imaging of susp. source	According to diagnostic imaging and clinical judgment	1. Cefuroxim 1500 mg x 3 i.v. or Ampicillin 1g x 4 / 2 g x 3 i.v. 2. Ciprofloxacin 400 mg x 2 i.v. 3. Consider: Metronidazol 500 mg x 2 i.v.
CATEGORY 2	• Blood culture • Tracheal secretion • Urine culture • Culture from susp. source • Diagnostic imaging of susp. source	According to diagnostic imaging and clinical judgment	1. Pip/Tazo[d] 4gx3 iv or Meropenem 1gx3 iv 2. Ciprofloxacin 400 mg x 2 i.v. 3. Metronidazol 500 mg x 2 i.v. 4. Consider fungal infection: Fluconazole i.v. and cath. inf: Vancomycin, dosage acc.to. Se-Vanco[e]
CATEGORY 3	• Blood culture • Tracheal secretion • Urine culture • Culture from susp. source • Diagnostic imaging of susp. source • Renewing oldest diagnostic imaging of susp. source	According to diagnostic imaging and clinical judgment	1. Pip/Tazo[d] 4gx3 iv or Meropenem 1gx3 iv 2. Ciprofloxacin 400 mg x 2 i.v. 3. Metronidazol 500 mg x 2 i.v. 4. Fluconazol 400 mg x 2 i.v. 5. Vancomycin, dosage acc.to. Se-Vanco[e]
CATEGORY 4	Nothing further	Standard-of-care approach	Continue present treatment
CATEGORY 5	Nothing further	Standard-of-care approach	Re-consider the indication for antibiotics (standard-of-care principle)

[a] 'Alert PCT' is defined as PCT-day1 ≥ PCT day 0 x 0.9. So a decrease in PCT from 11,2 ng/ ml to 10,5 ng/ ml is an "irrelevant decrease" and is defined as an "Alert" PCT. [b]DDD = Defined Daily Dosages). N.B.: The mentioned dosages are examples. Dosing regimen and frequency is prescribed according to the department guidelines (according to weight, kidney function, haemodialysis, Continuous dialysis etc.). [c]Antimicrobial spectrum covered can be broader than suggested (discretion of investigator). Administration of antimicrobials with a narrower spectrum on Alert-PCT days, should only take place when any antimicrobial treatment covering the suggested spectrum is contra-indicated and such a therapy should always be discussed and accepted by the coordinating centre. [d]Pip/Tazo: piperacillin/tazobactam. [e]Se-Vanco: serum-vancomycin measurements

Diagram D2:
Meta-analysis of randomized trials using piperacillin-containing regimens exploring renal failure



Results:

- In the initial identification phase, four ICU studies were found: They were excluded, since A) only a (non-defined) part of the patients received piperacillin(8), B) Both groups received piperacillin(9), C) one or both groups received aminoglycosides concomitantly(10, 11) .
- In the 7 (non-ICU) trials eventually included, 1592 episodes of therapy were observed.
- 21 cases of renal failure (not defined) occurred, corresponding to 1.3%.
- Hypothesizing, that the incidence of renal failure is 0.5% in non-piperacillin containing beta-lactam therapies, and aiming to find a risk increase to totally 1.5% (relative risk of 3.0), using conventional type I risk limit of 5% and a power of 80%, the sample size for such a trial investigating this should be approx. 3300 patients (non-ICU setting).
- In an ICU setting, the incidence of renal failure is often >20%. A trial of 1000 patients would be able to detect a risk increase to 28% (Relative risk:1.4) from e.g. piperacillin

*All articles were reviewed for this. Additionally, in adobe documents with the search option (those not scanned), a search was made in each pdf document with search terms: "renal", "kidney", "nephro", "creatinine" and "gfr". More than the noted 25 of the articles did not report renal failure, however, if they fulfilled one or more of the other exclusion criteria, they were excluded because of this.

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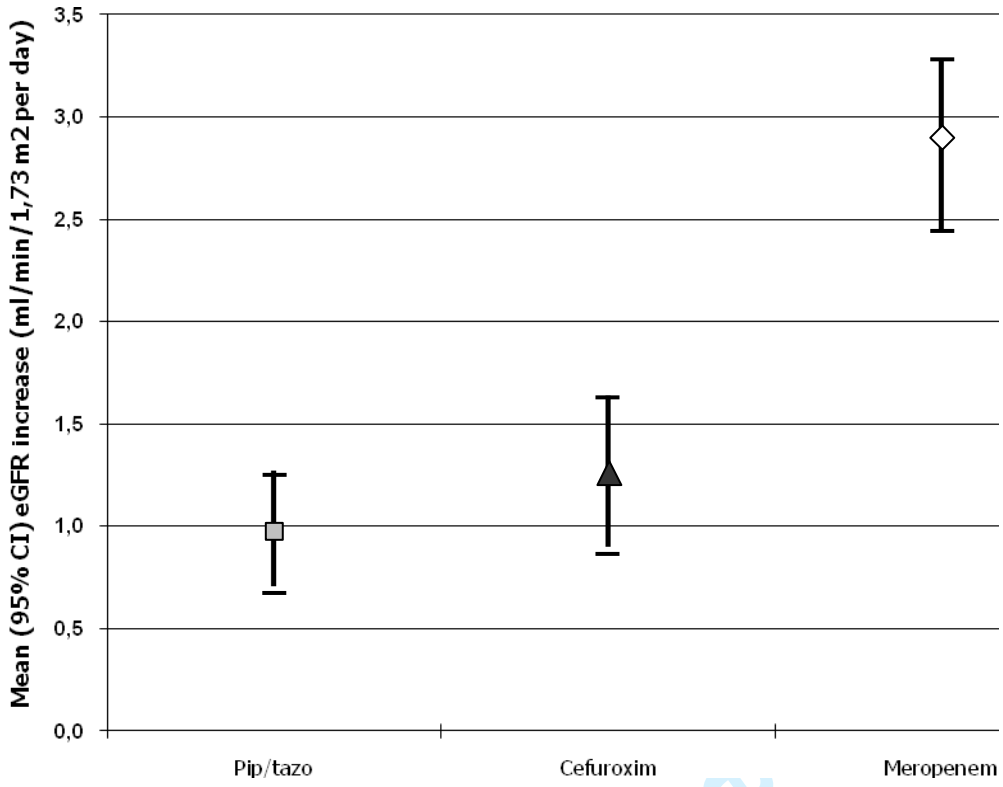

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CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts[21 31])	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	1,5,15
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6 + fig. 2 + Diagram D1
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	7-8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5

Section/Topic	Item No	Checklist item	Reported on page No
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6
	11b	If relevant, description of the similarity of interventions	6
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	6-7
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	6-7
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1 (CONSORT diagram
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1 (CONSORT diagram
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	8
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	8-9, table 3 +table 4
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect	

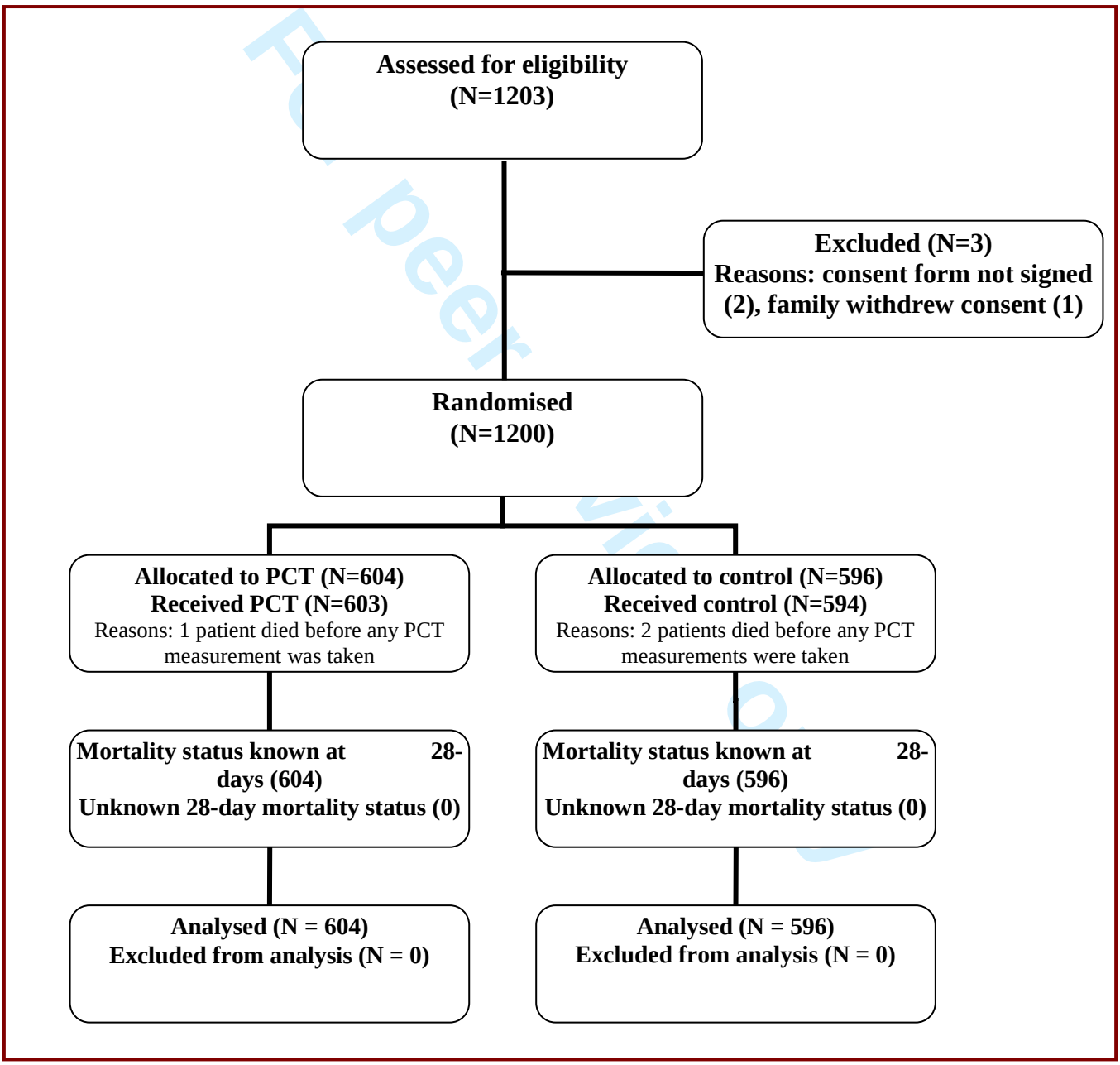
Section/Topic	Item No	Checklist item	Reported on page No
		size and its precision (such as 95% confidence interval)	9-10 + table 2, 3, 4 + fig. 3+4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Abstract + p.
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Table 3, fig. 3+4, p 10.
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms[28])	Table 3+4, p. 10-11, fig. 3+4
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	10-14
Other information			
Registration	23	Registration number and name of trial registry	4-5
Protocol	24	Where the full trial protocol can be accessed, if available	5
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration[13] for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials,[11] non-inferiority and equivalence trials,[12] non-pharmacological treatments,[32] herbal interventions,[33] and pragmatic trials.[34] Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

PASS-II 25th Aug 2010

Antibiotics and Renal Organ Failure – secondary endpoints from the Procalcitonin And Survival Study - analysis plan

1. Consort Flow Diagram (done in PASS-1)



Trial profile.

2. Baseline characteristics

Table 1: Baseline characteristics

	Standard-of-care-only n=596)	Procalcitonin-guided n=604)	Overall n=1200)
Age (Yr.) Median (IQR)	67 (58–75)	67 (58–76)	67 (58–76)
Male sex – no. (%)	333 (55·9%)	330 (54·6%)	663 (55·3%)
Body Mass Index – Median kg/m2 (IQR)	24·7 (22·0–27·8)	25·0 (22·5–28·7)	24·8 (22·2–27·9)
APACHE II Score - Median (IQR)	18 (13–24)	18 (13–25)	18 (13–24)
Surgical patient – no. (%)	260 (43·6)	227 (37·6)	487 (40·6)
Chronic co-morbidity* - no. (%)			
No chronic co-morbidities	102 (17·1)	123 (20·4)	225 (18·8)
1 chronic co-morbidities	279 (46·8)	257 (42·6)	536 (44·7)
2 chronic co-morbidities	173 (29·0)	171 (28·3)	344 (28·7)
≥3 chronic co-morbidities	42 (7·1)	53 (8·8)	95 (7·9)
Acute illness/reason for admittance to ICU – no. (%)			
Central nervous system incl. Unconsciousness	78 (13·1)	101 (16·7)	179 (14·9)
Respiratory failure	422 (70·8)	410 (67·9)	832 (69·3)
Circulatory failure	263 (44·1)	257 (42·6)	520 (43·3)
Gastro-intestinal disease	128 (21·5)	96 (15·9)	224 (18·7)
Renal disease	81 (13·6)	103 (17·1)	184 (15·3)
Post-operative complications	123 (20·6)	106 (17·6)	229 (19·1)
Trauma	113 (19·0)	106 (17·6)	219 (18·3)
Other	68 (11·4)	57 (9·4)	125 (10·4)
Indicators of severity			
Temperature, ^{0}C (median (IQR), n=1136)	37·3 (36·3–38·1)	37·4 (36·4–38·3)	37·3 (36·3–38·2)
Mean arterial pressure, mmHg (median (IQR) n=1195)	71 (60–84)	72 (63–85)	71 (62–84)
Heart frequency (median (IQR) n=1197)	100 (82–116)	100 (84–117)	100 (83–117)
Need for vasopressor/inotropic drug† (%, n=1200)	315 (52·9)	326 (53·4)	641 (53·4)
PaO2 /PaCO2 ratio (median (IQR), n=1178)	1·85 (1·27–2·62)	1·82 (1·29–2·53)	1·83 (1·28–2·59)
pH (median (IQR) n=1185)	7·29 (7·21–7·39)	7·29 (7·20–7·38)	7·29 (7·20–7·38)
Mechanical ventilation used (%, n=1200)	401 (67·3%)	401 (66·4%)	802 (66·8%)
Creatinine µmol/lL (median (IQR) n=1167)	119 (78–197)	119 (75–208)	119 (76–202)
Dialysis required (%, n=1200)	88 (14·8%)	86 (14·2%)	174 (14·5)
Bilirubin, µmol/L (median (IQR) n=1109)	10 (6–17)	10 (5–18)	10 (5–17)
Infection, clinical assessment ‡ – no. (%)			
No infection	118 (19·8)	86 (14·2)	204 (17·0)
Localized infection or Sepsis	266 (44·6)	271 (44·9)	537 (44·8)
Severe sepsis/ septic Shock	212 (35·6)	247 (40·9)	459 (38·3)
Site of infection § – no. (%)			
CNS	12 (2·0)	35 (5·8)	47 (3·9)
Respiratory	292 (50·0)	324 (53·6)	616 (51·3)
Gastrointestinal	149 (25·0)	145 (24·0)	294 (24·5)
Urinary	28 (4·7)	42 (7·0)	70 (5·8)
Other	52 (8·7)	41 (6·8)	93 (7·8)
Biomarkers			
Alert-PCT || – no. (%)	279 (47·0)	312 (51·7)	591 (49·4)
Leukocytes, x10^{9} – median (IQR)	13·0 (8·8–18·1)	12·4 (8·0–18·1)	12·8 (8·4–18·1)
C-reactive protein, mg/L – median (IQR)	152 (54–266)	161 (56–271)	157 (56–271)

Interquartile range (IQR). Acute Physiology and Chronic Health Evaluation II score (APACHE II) ranges from 0 to 71. *Chronic co-morbidity: Earlier diagnosed via hospital admission: heart failure, lung disease, cancer, diabetes, alcohol abuse, chronic infection, neurological disease, renal diseases, liver disease, gastro-intestinal disease, autoimmune disease, cancer and psychiatric disorders. Acute illness: persons can have several. 'Other' includes liver disease, haemorrhage, haematological disease and poisoning. †Vasopressors/inotropic drugs are considered to be epinephrine, nor-epinephrine, dopamine and dobutamine. ‡ Infections were rated according to the ACCP/SCCM definitions; investigators were trained in using them. § Site of infection: patients can have more than one. ||Alert-PCT: Procalcitonin-level not decreasing by at least 10% from the previous day and above 1·0 ng/ml. If only one measurement is available: Absolute procalcitonin-level above 1·0 ng/ml.

Table 1. Baseline characteristics of the study participants.

Table 2: Follow up characteristics

Follow up measurement	Control group (N=596)	PCT-guided group (N=604)	Overall (n=1200)
Patients followed and alive for 28 days (N., %)			
Patients followed for 28 days (incl. those who died in the first 28 days) (N., %)			
Status at 28 days (n =): Alive Dead			
Days spent in ICU Median (IQR) (as in PASS-I)			
Days spent in Danish hospital within 28 days Median (IQR)			
Patients with a complete 28 day follow up for respiratory failure (mech. Vent., PaO2 and FiO2)			
Days followed within 28 days for respiratory failure (mech. Vent, PaO2 and FiO2) of total days in trial ((denom. = 604 x 28) this can be drawn from the admission list in combination w. database)			
Patients with 28 day follow up for renal failure (dialysis – same as prev.)			
Days followed within 28 days for renal failure (dialysis) of total days in trial (denominator = 604 x 28 and 596 x 28 days) (same as prev.)			
Patients with 28 day follow up for renal failure (eGFR)			
Days followed within 28 days for renal failure (eGFR) of total days in trial (denominator = 604 x 28 and 596 x 28 days)			
Patients with 28 day follow up for Platelets			
Patients with 28 day follow up for Bilirubin			
Patients with 28 day follow up for antibiotic consumption			

n*s refers to the total number of patients who had follow up for 28 days.
28-day follow up is: Follow up until death within 28 days OR until day 28. For respiratory failure follow up is done for all ICU admissions. For renal failure, follow up is done for all dialysis treatment (ICU+other dialysis competent hospital units) and for all creatinine and carbamide measurements performed within 28 days (ICU + non-ICU admissions). For platelets and bilirubin, follow up is done for all measurements performed within 28 days (ICU + non-ICU admissions)

STRATIFICATION (*S) / test for interaction: (regarding the below analyses in Section 2 + 3)

1. **Age (limit initially 65 y, if significant interaction, more age groups**
2. **APACHE II score (limit initially 20, if significant interaction, more APACHE II groups,**
3. **Site 1-9.**
4. **Severe Sepsis/septic Shock vs. Milder or No infection at Baseline**
5. **Calendar date of inclusion into PASS. Recruited: 9th Jan 2006 – 31st December 2007 (~430 patients) vs. 1st of Jan 2008 – 2nd of June 2009 (~770 patients).**
6. **Surgical patient / medical patient [Surgical = All patients with mark in Baseline "B6", or "B12" or marked "Yes" in "L"]**
7. **Gender**

SECTION 2. Exposure – Antibiotic usage

Follow up: All patients were followed up regarding antibiotic consumption: 1) In the ICU in the primary PASS-CRF, 2) All ICU-surviving patients, not staying in the ICU for 28 days, were followed up for antibiotic consumption in the non-ICU, they were discharged to after ICU.

General: The aims of these analyses are to investigate the impact of performing PCT-guided empiric antibiotic interventions according to a progressive algorithm on the consumption of antibiotics. This is to be illustrated by analyses exploring 1) spectrum, 2) quantity and 3) duration of therapy in the two arms.

The aim is:

a) To investigate the difference in exposure in general to antibiotics in the two arms of the PASS trial and more specifically to broad-spectrum antibiotics.

This is done in the following analyses (PCT vs. Control):

1) The total number of days within the 28 day follow-up period with any antibiotic treatment (or proportion of follow-up time): [Not done Yet]
2) The total consumption of any antibiotic in weight (grams within 28 days) [Not done Yet]
3) The total consumption per ICU day of any antimicrobial [DONE]
4) The total consumption of betalactam drugs active against most Extended Spectrum Beta-lactamases and wild-type Pseudomonas aeruginosa (a. Meropenem and other pseudomonas active carbapenems, OR b. Piperacillin/tazobactam OR c. 4.generation Cephalosporins). [or proptortion of days in these treatments] [Not done Yet]
5) The total no. of days within the 28-day follow up period with treatment with any flour-quinolone (ciprofloxacin, moxifloxacin and others) [or proptortion of days in these treatments] [Not done Yet]
6) The total no. of days within the 28-day follow up period with treatment with any glycopeptide (Vancomycin, Teicoplanin) [or proptortion of days in these treatments] [Not done Yet]
7) The total no. of days within the 28-day follow up period with treatment with fluconazole [or proportion of days in these treatments] [Not done Yet]

Consumption of antimicrobials in the intensive care unit				
Length of antimicrobial treatment in ICU, days (median, IQR)	4 (3– 10)	6 (3– 11)	-	0·001
Quantity of antimicrobials administered per ICU day (g) (median, IQR)	6·7g (4·5g– 12·5g)	8·6g (5·3g– 13·7g)	-	<0·001
Number (%) ICU days spent with at least three antimicrobials	2721 (57·7%)	3570 (65·5%)	-7·9% (-9·7%– -6·0%)	0·002

*Counted from the time of sampling. Only samples later to become positive. Cultures with coagulase negative staphylococci, corynebacteria and propionebacteria are not included. † Including localised infection, mild sepsis, severe sepsis and septic shock. p-values for the number of days spent with each factor were generated by testing the proportion of intensive care days spent with each factor using non-parametric tests. ICU: Intensive care unit

Table 3. Antibiotic consumption

Admission time within 28 days

1. Number of days admitted to hospital within 28 days after recruitment. Median + IQR. (PCT vs. Control)

Subgroup Analysis: Total use of Antimicrobial chemotherapy

1. Total antibiotic prescription days (all AMCs received, where all AMCs are weighted equally and summed per day, e.g.:→ possible to have e.g. 30 prescription days in 10 days ICU)

Table 3: Number of AMCs received per day (over all days)

	PCT-arm	Control-arm	P-value
AMC total (N,. %)			
Recruited 09/01/06 – 31/12/07 Recruited 01/01/08 – 02/06/09			
Age <65 years Age ≥65 years			
APACHE II <20 APACHE II ≥20			
Bispebjerg Gentofte Glostrup Herlev Hillerød Hvidovre Roskilde Skejby Århus			
Severe Sepsis or septic shock at BL Milder or no infection at BL			
Surgical patient Non-surgical patient			
Gender			

MICROBIOLOGY

Follow up: All patients were followed up via the electronic registers at the microbiologic depts., who service the PASS-ICU´s regarding all microbiologic samples performed from baseline and until 28 days after. Data have been merged in the PASS-database.

Table 4: Number of culture samples performed within 28-days from randomisation [Not done Yet – JU handles this]

Intervention		PCT arm N =	Control Arm N =	P-value
Microbiology:	**N., (%)**			
Blood Cultures	N. Yes, (%)			
Urine Cultures	N. Yes, (%)			
Airway Cultures	N. Yes, (%)			
Samples from other foci	N. Yes, (%)			

SECTION 3a: Estimating the degree of Organ Failure (OF)

Follow up: All patients were followed up regarding respiratory failure (mech. Vent + physiologic parameters) and renal failure at 1) the PASS-ICU where the patient was recruited in the primary PASS-crf, 2) regarding mech. Ventilation and physiologic parameters and renal failure at any other PASS-ICU within the 28 day period (when patients were discharged to such an ICU, 3) in the case that a patient was discharged within the 28 day period to a non-PASS ICU (seldom), follow up was made for mech. Vent. and physiologic parameters and renal failure in hospitals "Rigshospitalet" and "Bispebjerg", since only very few ICU days were spent at any other ICU within the 28 day period (48 days of approx 9900 days = approx 0.5%).

The purpose of these analyses is to explore in detail, the quantity of the occurrence of secondary endpoints in the PASS-trial, especially respiratory organ failure and renal organ failure.

Genuine hypothesis: High usage of broad spectrum antibiotics as used in the PASS trial, results in substantially reduced organ function (respiratory, renal and liver) and compromised coagulation and a likewise substantially increased time with manifest organ failure as defined clinically (need for organ support) AND biochemically/fysiologically (measured objective parameters).

NB: Analyzes are summarized in the table 5 below

time)

A. Renal Failure:

a. Median/ Mean eGFR for day1 – day10
b. Median/ Mean eGFR for day11 – day28
c. Median/Mean eGFR for day1 – day28 (a+b) [eGFR on days in columns in a figure and AUC for the columns]
d. Median/Mean Carbamide for day1- day10
e. Median/ Mean Carbamide for day11 – day28
f. Median/Mean Carbamide for day1 – day28 (a+b) [Carbamide level on days in columns in a figure and AUC for the columns]
g. Median/Mean Platelet count for day 1-28 [[platelet on days in columns in a figure and AUC for the columns]
h. Median/Mean Bilirubin [Bilirubin on days in columns in a figure and AUC for the columns]
i. No. of days within 28 days with eGFR < 60 ml/min/1.73 m2
j. No. of days within day1 – day10 with eGFR < 60 ml/min/1.73 m2
k. No. of days within day1 – day10 with dialysis
l. No. of days within day11 – day28 with dialysis
m. No. of days within day1 – day28 with dialysis

C + F+ G + H are all part of one figure with 4 panels.

Explanations: A: Dialysis:

Patients are categorized on days with ND or NA as dialysis=0, since this means patient has been discharged to home. All admissions within 28 days have been drawn from the central hospital register (Green System) and all admissions at dialysis capable departments have been followed up with dialysis.

B: eGFR:

In the ICU, patients are categorized with a new eGFR every day (done in PASS).

Patients are categorized on the basis of their status of eGFR on the last day of ICU. This status is kept until a creatinine measurement is done (on which day the status is changed to a new eGFR). This status is then kept until the next time creatinine is measured – and so forth. In this way every day from 1 – 28 is given an eGFR status.

In summary, the same principle is used: From day 1, the first time a creatinine is measured, a eGFR is calculated. Next time the patient has a creatinine measurement, the patient is re-categorized with a new eGFR. That eGFR is kept until the next creatinine measurement etc.

Table 5. Prevalence and duration of organ failure and other severe disturbances (PCT vs. Control)

	PCT arm (n =)	Control Arm (n =)	P-value
Kidney Failure mL/min/1.73 m^2 (N. days, % of total days): Normal: GFR > 90 Mildly impaired: 60–89 Moderately/severely impaired: GFR <60			
Kidney Failure Median/ Mean eGFR for day1 – day10			
Kidney Failure Median/ Mean eGFR for day11 – day28			
Kidney Failure Median/Mean eGFR for day1 – day28 (a+b)			
Kidney Failure Median/Mean Carbamide for day1- day10			
Kidney Failure Median/ Mean Carbamide for day11 – day28			
Kidney Failure No. of days within 28 days with eGFR < 60 ml/min/1.73 m2			
Kidney Failure No. of days within day1 – day10 with eGFR < 60 ml/min/1.73 m2			
Kidney Failure No. of days within day1 – day10 with dialysis			
Kidney Failure No. of days within day11 – day28 with dialysis			
Kidney Failure No. of days within day1 – day28 with dialysis			

Table with summarized analyses.

SECTION 3b: Attempting to explain the reason for organ failure (if OF is confirmed in section 3a)

Antimicrobial toxic explanation

Genuine hypotheses:

1) High Exposure (at least 5 or at least 10 days) to a certain combination of antibiotics (Pip/Tazo+Cipro OR Meropenem + Cipro OR Pip/Tazo + Vanco OR Meropenem + Vanco) causes OF

For 2-6: Estimate accumulated risk for day 1, 2, 3 etc. separately in both PCT group and control group.

2) Treatment for more than 4 days with Pip/Tazo causes OF (also 10 days)
3) Treatment for more than 4 days with Ciprofloxacin causes OF (also 10 days)
4) Treatment for more than 4 days with Meropenem causes OF (also 10 days)
5) Treatment for more than 4 days with Vancomycin causes OF (also 10 days)
6) Treatment for more than 4 days with Cefuroxim causes OF (also 10 days)

For the below analyses two composite endpoints are used for the Pulmonary/renal OF:

1) **Organ failure endpoint A**: Clinical Organ Failure judgment: Endpoint=1 for any day with dialysis. If both are present, Endpoint=2. Results are presented as “Clinical Organ Failure Days”
2) **Organ failure endpoint B:** Objective Organ failure measures: Endpoint =1 for any day with eGFR <30, repeated with <60 ml/min/1,73 m2. “Objective Organ Failure Days”

Analyses:

A. Objective Organ failure endpoint:

As above, 1) – 6).

1) Analyze the median “Objective Organ Failure Days” to occur from “P-T treatment day 5” until 10 days later (counting endpoints for next 10 days). Censor at death.
2) Analyze the median “Objective Organ Failure Days” to occur from “Meropenem treatment day 5” until 10 days later (counting endpoints for next 10 days). Censor at death

B. Multiple Effects models:

Regarding renal dysfunction: Analyze renal recovery in eGFR progression per day on different drugs day 1-10 (Meropenem / Piperacillin-tazobactam / Ciprofloxacin / Cefuroxim), control for other known predictors of renal failure. Additionally after discontinuation of these drugs.

Sensitivity analyzes:

Cox or Logistic Regression ?

Endpoint: Binary endpoint. To be defined according to the median number of organ failure days within 10 days after exposure for 5 days.

Endpoint 1a: [>median number of “clinical organ failure days”]

Endpoint 1b: [>median number of “clinical organ failure days”+2 days]

Endpoint 2a: [>median number of “objective organ failure days”]

Endpoint 2b: [>median number of “objective organ failure days”+2 days]

Risk variables to be entered:

a. Treatment for >=4 days with Pip/tazo
b. Treatment for >=4 days with Meropenem
c. Treatment for >=4 days with Ciprofloxacin
d. Treatment for >=4 days with Vancomycin
e. Treatment for >=4 days with Pip/tazo + Ciprofloxacin (all 4 days)
f. Treatment for >=4 days with Meropenem + Ciprofloxacin (all 4 days)
g. Treatment for >=4 days with Pip/tazo + Vancomycin (all 4 days)
h. Treatment for >=4 days with Meropenem + Ciprofloxacin (all 4 days)
i. Treatment for >=4 days with Meropenem + Vancomycin (all 4 days)
j. APACHE II >=20
k. Age >=65
l. Surgical patient
m. Severe sepsis/septic shock

NB: Treatment count start days 1 – 13 (so 5 days complete on day 5 – 18).

Patients with pauses in the administration of >=1 day → exclude

Only count the first administration

Endpoints:

“Clinical Organ Failure Days” and “Objective Organ Failure Days” both as defined above

→Transformed to Binary endpoint:

Endpoint 1a: [>median number of “clinical organ failure days”]

Endpoint 2a: [>median number of “objective organ failure days”]

(as above in the sensitivity analysis)

PASS-II, organ failure – authors,

Forfattere

Chip: JU+JDL+LRN

KMA Hvh/Diacenter: BEL

Glostrup: Mulige: Asger, Anne, Ditte

Hvh: Mulige: Peder C, Jesper, Morten

Herlev: Mulige: Peter, Hamid, Tina

Gentofte: Mulige: Thomas, Katrin

Hillerød: Mulige: Morten, Lars, Kristian A?

Roskilde: Mulige : Niels-Erik

Århus: Mulige: Kim + Mads

Skejby: Mulige: Paul

Protocol

A randomised, single-blinded, multicentre trial to investigate if clinical management guided by daily standardised Procalcitonin measurements can reduce the mortality in critically ill patients

The Procalcitonin and Survival Study (PASS)

Version of protocol: 3.1

Date: December 2006

Intensive Care Units from many University Hospitals all over Denmark will participate:

Sponsor: Scientific:
Copenhagen HIV Programme (CHIP) 044, Hvidovre University Hospital, Denmark
: Economic: Danish Research Council (Danish State) and other independent research foundations

Protocol co-ordinator

Jens-Ulrik Stæhr Jensen
H:S Hvidovre University Hospital
DK - 2650 Hvidovre
Denmark
Phone: +45 36 32 33 07
Fax: +45 36 47 33 40
E-mail: koordinator@pass-studiet.dk

INVESTIGATOR PROTOCOL AGREEMENT PAGE

THIS AGREEMENT IS EQUIVALENT TO A "SIGNED PROTOCOL"

The PASS Trial

Name and qualifications of investigator:

Name of Investigator: _____

Post held: _____

Clinical Centre: _____

I agree:

- to assume responsibility for the proper conduct of the PASS Trial at this site.
- to conduct the trial in compliance with this protocol, any future amendments, and with any other trial conduct procedures provided.
- not to implement any deviations from or changes to the protocol without agreement from the sponsor and prior review and written approval from the Independent Ethics Committee (IEC), except where necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the trial (where permitted by all applicable regulatory requirements).
- that I am thoroughly familiar with the appropriate use of the Procalcitonin test and the interpretation of the test results, as described in this protocol, and any other information provided by the manufacturer of the test and by the PASS Coordinating centre.
- that I am aware of, and will comply with, "Good Clinical Practice'' (ICH-GCP Guideline (CPMP/ICH/135/95, Directive 2001/20/EC)) and all applicable regulatory requirements.
- to ensure that all persons assisting me with the trial are adequately informed about the Procalcitonin test and interpretation and of their trial-related duties and functions as described in the protocol.

_____ _____

Signature of investigator Date

One signed copy each to be held by the Investigator and PASS Co-ordinating centre.

15/10/2007

TABLE OF CONTENTS

A randomised, single blinded, multicentre trial to evaluate whether daily Procalcitonin measurements and immediate diagnostic and therapeutic response on abnormal values and day-to-day changes can reduce the mortality of critically ill patients in the Intensive Care Unit.

The Procalcitonin And Survival Study (PASS)

PROTOCOL SUMMARY

Inclusion:

Fulfilment of all of the following three criteria:

1 Male or female, aged ≥ 18 years of age.

2 Admitted to the participating intensive care units (ICU) at following hospitals: Hvidovre Hospital; Bispebjerg Hospital; Herlev Hospital; Glostrup Hospital; Gentofte Hospital; Hillerød Hospital; Roskilde Hospital; Århus University Hospital, Århus; Århus University Hospital, Skejby.

3 1) Ability to understand and provide written informed consent to participate in this trial,

or

2) Ability to understand and provide oral informed consent in presence of at least one impartial witness who should sign and personally date the consent form

or

3) The subjects legally acceptable representative can understand and provide written informed consent if the subject is not capable of this because of the present mental or physical condition of the subject.

Exclusion:

A subject will **NOT** be eligible for inclusion in this trial if any of the following criteria apply:

1. Subjects with known hyper-bilirubinaemia (>0.4 mg/ ml) or hypertriglyceridaemia (>10 g/l) since this can interfere with measurements. If subjects with unknown status on these points are included and have PCT measurements, the measuring-equipment will detect these conditions.

2. Subjects suffering from a blood disorder, where daily sampling of 7 ml of blood for maximally 28 days (210 ml distributed on 28 days) will be an inconvenience or a potential risk, which could compromise the safety of the subject.

3. Subjects who are pregnant or breast feeding

The *a priori* probability of surviving with the normal recommended diagnostics and treatment with the presently available means to detect infections and on the other hand the normal diagnostics and treatment together with daily Procalcitonin measurements and prompt clinical reaction should be equal.

Randomisation:

Two arms (1:1), n = 500 per arm:

Arm 1: Normal recommended diagnostics and treatment of infections in the intensive care unit (standard of care)

Arm 2: Normal recommended diagnostics and treatment of infections in the intensive care unit (standard of care) **and** Procalcitonin guided diagnostics and treatment of infection

Primary Trial Objective: To address whether daily Procalcitonin measurements and immediate diagnostic and therapeutic response on abnormal values and day-to-day changes can reduce the mortality of critically ill patients in the ICU.

Trial registration days: Intensive Care Unit admission day, running routine registration of examinations and blood tests, day of discharge or death, day 28 after admission, day 60, 90, 120 and 180 after discharge.

Data collection: The data collection will be simple and performed real time via fax.

1 TRIAL BACKGROUND AND RATIONALE

1.1 Background

1.1.1 *Sepsis and mortality in the Intensive Care Unit*

Sepsis remains a major cause of mortality in critically ill patients admitted to the Intensive Care Unit (ICU) [1-2]. All-cause mortality during ICU admission ranges from 12.1% in non-infected patients to 43.9% in infected patients[3]. Patients who are discharged to other departments and later to their own home or an institution for rehabilitation, continue to have a high mortality (additionally 10-20%) for 20-30 days after ICU discharge[4-7]. Different explanations for this have been proposed. Among the most important are:

1) During ICU admission it becomes clear that further treatment lacks perspective for the patient (often chronical organ diseases and cancer diseases) and the patient is therefore discharged to the relevant department when discharge from the ICU is possible.
2) After discharge from the ICU the physical condition of the patient deteriorates because of a severe disease with a dismal prognosis and it is decided together with the patient and relatives that the patient should not be admitted to the ICU again.
3) Critically ill patients often have an immunological incompetence and therefore these patients are susceptible to serious infections. Additionally these infections often have an atypical course and thereby a delayed diagnosis. This immunological incompetence prevails some time after discharge from the ICU why the patient remains susceptible to infections for this period of time. There is a grave risk that these serious infections with an atypical course can be diagnosed late in the course and cause an increased risk of mortality for critically ill patients.

1.1.2 *Procalcitonin and bacterial infections*

In 1993 Assicot et al. reported that a high level of serum-Procalcitonin (PCT) was closely related to bacterial infection and seemingly correlated to the severity of the infection[8]. This finding has since been ascertained in many studies demonstrating high levels (2.0 ng/ml-50.0 ng/ml (-1500 ng/ml)) of PCT in patients with systemic bacterial infection, while low levels have consistently been found in patients with localised bacterial infections and viral infections[9-16]. Others have shown low PCT levels (and seldom up till maximally 3.0 ng/ml) in non-infected patients following surgery, trauma and myocardial infarction[10, 17-21]. Sensitivity and specificity for sepsis when PCT levels are above 5.0 ng/ml have been estimated to 80-90 % and 85-100%, respectively, in the largest of these studies.
The PCT level starts decreasing within 24 h after surgery, trauma and myocardial infarction in non-infected patients in contrast to the C-reactive protein, which has a peak level 36-72 h after these events[10-17-21].

Consequently, bacterial infection is suspected if PCT is increasing 24 h after surgery, trauma or myocardial infarction.

1.1.3 *Procalcitonin kinetics, biochemistry and cellular biology*

PCT is a 13 kDa, 116 amino acid polypeptide, initially described as a pro-hormone of Calcitonin, a

hormone in the calcium metabolism, which is produced in the medullary C-cells in the thyroid gland[22-24]. Recent studies have shown that the PCT variant, which is related to infection is produced in other tissues (liver, kidney, muscle, fat)[25-27]

Kinetic studies with healthy humans and baboons have shown a rapid release of PCT within 2-6 hours after injection of bacteria or bacterial endotoxin. This time to release is significantly shorter than that of C-reactive protein (8-24 h). The plasma half life of PCT is approximately 24 h. PCT measurements in healthy, uninfected volunteers has been shown very low levels (<0.05 ng/ml)[10,28-29].

1.1.4 *Procalcitonin-guided treatment and reduction in the use of antimicrobial agents*

A recent study has demonstrated a reduced use of antimicrobial agents in patients with lower respiratory tract symptoms, when the treatment was guided by the initial PCT level[30].

1.1.5 *Procalcitonin and risk of mortality*

We have shown that a PCT increase after reaching a level of 1.0 ng/ml is an independent predictor of mortality in critically ill patients. Patients who did not reach a PCT level above 1.0 ng/ml had an all cause mortality risk of 4.7% while admitted in the ICU, compared to an all cause mortality of 19.1% for the whole population of ICU patients. Patients who reached a PCT value above 1.0 ng/ml who had a decreasing PCT the next day had a mortality risk of 18.9%, but patients who had an increasing PCT level after reaching 1.0 ng/ml had a mortality risk of 32.7%. This increase in mortality risk was significant for the entire follow-up period of 90 days[31].

The mortality risk increased for every day the PCT increased. Taking in mind the close relation between PCT levels and bacterial infection, a large part of this mortality increase is (when PCT is increasing), to the best of the existing knowledge, attributable to uncontrolled bacterial infections. This is supported by the findings of the European Sepsis Group[3].

The rapid release of PCT to the blood stream (2-6 h), when infection is progressing, makes acute detection of ongoing serious infection possible, hereby potentially reducing mortality in critically ill patients if treatment is guided acutely by PCT measurements.

1.2 Rationale - summary

Sepsis and complications to sepsis are major causes of mortality in critically ill patients[1-2]. Rapid treatment of sepsis is of crucial importance for survival of patients. In the ICU, the infectious status of the patient is often difficult to assess because symptoms cannot be expressed (unconscious or sedated patients) and signs may present atypically because of immunologic incompetence and masking by the drugs given and thermo-influencing-therapy, i.e. dialysis. Biological and biochemical markers of inflammation (WBC, C-reactive protein) may often be influenced by other parameters than infection, such as: trauma, surgery, other types of inflammation such as rheumatoid diseases (C-reactive protein) and gluco-corticosteroid treatment (WBC), and may be unacceptably slowly released after progression of an infection[32-33]. At the same time, lack of a relevant antimicrobial therapy in an early course of infection may be fatal for the patient.

For these reasons, in the clinical setting, it is often necessary to initiate or adjust antimicrobial therapy on an unsure ground and the relevant therapy may in some situations be delayed for important hours or even days. Specific and rapid markers of bacterial infection have been sought for use in the ICU. Mortality in critically ill patients increases gravely when Procalcitonin levels increase from day to day[31]. Low PCT levels have been shown to effectively rule out sepsis[12].

However, no randomised controlled trials have been conducted to show if mortality in critically ill patients can be reduced by using a strategy of daily standardised Procalcitonin measurements as an early detector of serious bacterial infection. Therefore evidence is presently not sufficient to introduce daily consecutive Procalcitonin measurements to guide the diagnostic and therapeutic management of patients admitted to the ICU .

The rationale for this trial is to assess the ability of daily Procalcitonin measurements to reduce the mortality of critically ill patients.

1.3 Procalcitonin analysing methods

There are four commercially available analysing methods for measuring blood levels of Procalcitonin, one semi-quantitative and three quantitative. Two of these are described below, the oldest and most used test, *LUMITEST ® BRAHMS /BRAHMS PCT LIA,* and a newer fully automated test with a higher sensitivity, *KRYPTOR® PCT BRAHMS.* KRYPTOR® PCT BRAHMS will be used for all Procalcitonin analyses in this study[34].

1.3.1 LUMITEST ® BRAHMS /BRAHMS PCT LIA

The oldest and so far most used quantitative test is LUMITEST ® BRAHMS /BRAHMS PCT LIA. Analysis is made by a ”sandwich” luminiscens immuno-assay with an anti-catacalcin coated tube:
Anti-**Catacalcin** binds catacalcin in the patient sample and is hereby immobilised (catacalcin could otherwise interfere with the analysis).
Anti-**Calcitonin** antibody is marked with a luminescent a*cridin*-derivative.
H_2O_2 and NaOH are added and these react with the *acridin*-derivative which leads to the formation of *acridon* and this process is accompanied by transmission of light. The quantity of this light is proportional to the Procalcitonin concentration in the sample.
We have found a coefficient of variation (CV) in the measuring interval between 0.1 ng/ml-1.0 ng/ml of 0.09-0.83 for this test. At PCT levels above 1.0 ng/ml, we found CV´s of 0.008-0.065 (range)[37].
The manufacturer claims a *functional assay sensitivity* (CV<0.2) of 0.3 ng/ml.

1.3.2 KRYPTOR® PCT BRAHMS

A new, and according to the manufacturer, more precise assay is the fully automated KRYPTOR® PCT BRAHMS. Procalcitonin is analysed using the analysing machine KRYPTOR® and fluids and utensils from the company BRAHMS diagnostica, Berlin. KRYPTOR® uses

TRACE technology (Time Resolved Amplified Cryptate Emission), which is a non-radiating transmission of energy. The transmission happens between two flourescent compounds: Europium Cryptate (donor) and XL665 (acceptor). While the antigen-antibody complex is formed, a signal is measured.

The functional assay sensitivity (CV< 0.2) is according to the manufacturer 0.06 ng/ml for the KRYPTOR ® test. In the relevant clinical interval (which has not quite been defined yet) the CV is 0.02-0.03 (product information).

- Studies concerning Procalcitonin have so far mainly been using LUMITEST ® BRAHMS /BRAHMS PCT LIA.

1.4 Rationale for a 24 h interval between blood sampling

Several studies have shown a half-life of Procalcitonin of 20-30 hours and Procalcitonin levels increase 2-6 h after bacterial products are presented in the blood stream [10,28-29, 35]. An important exception to this is patients suffering from severe uraemia, where the Procalcitonin half-life is prolonged, but it has been demonstrated, that Procalcitonin is removed by dialysis[35]. Studies concerning Procalcitonin and surgery have shown, that the Procalcitonin blood level is on a decreasing curve 24 h after major thoracic and abdominal surgery, except in infected patients[17-21]. In conclusion, a Procalcitonin level which is increasing 24 h after a therapy shift or after surgery suggests progression of infection.

1.5 Procalcitonin and immuno-compromised patients

Markers and mediators of inflammation and infection are often dependent on a functioning immune system, which is able to produce the substance measured, e.g. WBC, TNF, different interleukins[10,15,16, 36]. It has been established that Procalcitonin is not dependent on blood cells and their mediators, and Procalcitonin is mainly produced by tissues like liver, kidney, muscle and fat[25-28]. In concordance with this, studies investigating Procalcitonin in neutropenic patients have found results comparable to those for immuno-competent patients[36-41]. A few studies regarding neutropenic patients that compared PCT levels to positive blood cultures have found a low sensitivity of the test for bacteriemia, but these studies lack clear definitions of virulence of different micro-organisms (e.g. Coagulase negative staphylococci vs. Gram negative rods) in their study designs[40].

1.6 Studies on Procalcitonin biology and bacterial infection

1.6.1 In vitro and animal studies

In vitro studies have shown Procalcitonin to be an inducer of albumin synthesis in rat liver tissue measured on mRNA and protein synthesis. This was found to be opposite to TNFα and IL-6, these substances lowering albumin synthesis[42]. In a study of sepsis in baboons, low PCT was

found in non-infected subjects and high PCT in infected subjects, and PCT blood levels started increasing after 2 hours[10]. In another baboon model Procalcitonin incompetence was shown in an anhepatic subject[28].
In a study of burn wound and Pseudomonas aeruginosa septicaemia in rats, a high correlation between endotoxin levels and PCT in blood was found[43].

1.6.2 Human observational studies

Most of the present knowledge on Procalcitonin has been established by observational studies. Key-references are mentioned in paragraph 1.1 and 1.2

1.6.3 Clinical trials

Only few Randomized Controlled Trials regarding PCT-guided treatment have so far been published, one of special interest has used PCT-guided treatment (n=119+124)and has assessed the ability of this clinical strategy to reduce use of antimicrobial therapy in patients with suspected lower respiratory tract infection. A Relative Risk of 0.49 [95% CI 0.44-0.55] for antibiotic exposure was demonstrated, without any significant difference in culture growth from patient samples, quality of life, mortality, inflammatory parameters (temperature, C-reactive protein, WBC), number of days admitted and need for stay in intensive care unit. The study was designed to detect a 30 % difference with 95% stringency. However some of the mentioned endpoints do not occur in all patients, and in these cases (mortality, need for stay in ICU) it may be false to conclude, that there is no difference between groups within the chosen 30 % limit[30].
A very small study (n=12+13=25) has tried to investigate empiric prophylaxis with fluor-quinolone Ofloxacin in patients with abdominal aortic aneurism. However the sample size of this study does not justify any conclusions on this issue[44].

2 TRIAL OBJECTIVES AND ENDPOINTS

2.1 Trial Objectives

2.2 Primary Objectives

To address whether immediate diagnostic and therapeutic initiatives guided by abnormal high and increasing values of Procalcitonin measured daily can reduce the mortality of critically ill patients in the ICU.

2.3 Secondary Objectives

1. To determine mortality of ICU patients at discharge from the ICU, at day 60,90, 120 and 180.

2. To determine differences in prescription of antimicrobial therapy in the two arms.
3. To determine the frequency of patients with complications to infection in the two arms, defined as; sepsis, severe sepsis, septic shock, disseminated intravascular coagulation, multi-organ dysfunction syndrome (MODS), coma (Glasgow Coma Scale), hypotension, respiratory insufficiency (ventilator treatment need), liver insufficiency, acute uremia (three times increase in baseline creatinine).
4. APACHE II score
5. Accumulated PCT increases over time
6. To determine the number of diagnostic image procedures per day after enrolment in the trial in the two arms
7. To determine the number of non-routine microbiological samples taken per day after enrolment in the trial in the two arms
8. To determine the number of surgical procedures per day after enrolment in the trial in the two arms
9. To determine the time to the first change in antimicrobial chemotherapy after admittance to the ICU in the two arms

2.4 Trial Endpoint(s)

Primary:

Mortality at day 28 after admission to the ICU.

Secondary:

1. Mortality while admitted to the ICU, Mortality at day 60, 90 and 180 after admission to the ICU
2. Defined day doses of antimicrobial therapy in each arm
3. Occurrence of sepsis, severe sepsis, septic shock, DIC. Assessment of Glasgow Coma Scale, measurement of Blood Pressure (systolic blood pressure < 90), days with artificial ventilation, Factor 2-7-9 < 0.7, creatinine (increase factor 3 from baseline), MODS.
4. SOFA score daily (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale).

5. $AUC_{Procalcitonin}$ for the Procalcitonin-measuring group and for the control group.
6. Number of diagnostic images after admission to the ICU.
7. Number of non-routine microbiological sample taken after admittance to the ICU.
8. Number of surgical procedures during the trial
9. Time to the first change in antimicrobial chemotherapy after admittance to the ICU

3 INVESTIGATIONAL PLAN

3.1 Trial Design

3.1.1 Intervention

This is a randomised, single-blinded multicentre trial.

Approximately 1000 subjects admitted to an ICU in the participating University hospitals will be included. All patients included will receive the the standard recommended diagnostic and therapeutic procedures mandated at the particular ICU. Additionally, the patients will be randomised for:

1. No PCT guided diagnostics and treatment (i.e. the standard-of-care / **control arm**).

Or

2. Daily PCT measurements and protocol-specified additional diagnostic and/or therapeutic interventions guided by the PCT levels observed. High or increasing PCT levels will mandate such interventions (see section 3.3.1 for details of interventions)(the **PCT intervention arm**)

3.1.2 Randomisation

The randomisation is performed by the PASS study centre and is stratified according to site, age and initial Acute Physiology And Chronic Health Evaluation II (APACHE II) score. For patients randomised to the PCT intervention arm, daily PCT levels are communicated to the team responsible for the clinical management together with a recommendation of what interventions the investigator team is expected to initiate based on the PCT measurement. In

the control arm, blood samples for PCT will be analysed simultaneously with samples from the PCT intervention arm, but results of these PCT analyses will remain blinded for the investigators until the study has been completed. The PCT measurements will be conducted daily as long as the patient is admitted to the ICU, but maximally 28 days from time of enrolment in this study. While patients remain in the hospital, and after discharge from the ICU, samples will be collected for PCT determination but the samples will not be analysed real-time and hence the results will not be used to guide interventions outside the ICU, except if requested by the ICU investigator in conjunction with the discharge of the patient. Patients transferred from one ICU to another ICU, will remain in the trial provided that the receiving ICU also participates in this trial.

3.2 Trial Population

3.2.1 Inclusion Criteria

A subject will be eligible for inclusion in this trial only if all of the following criteria apply:

1 Male or female, aged ≥ 18 years of age.

2 Admitted to the participating intensive care units. Patients should be included within 24 h. If a patient has not been included at this time, this patient cannot be included in the present admittance.

3 Subjects should in the investigator's opinion be likely to be admitted to the ICU for more than 24 h. Subjects should not be likely (<10%) to die or be discharged in this period of time

4 Ability to understand and provide written informed consent to participate in this trial,

or

Ability to understand and provide oral informed consent in presence of at least one impartial witness who should sign and personally date the consent form

or

The subjects legally acceptable representative can understand and provide written informed consent if the subject is not capable of this because of the present mental or physical condition of the subject.

3.2.2 Exclusion Criteria

A subject will **NOT** be eligible for inclusion in this trial if any of the following criteria apply:

4. Subjects with known hyper-bilirubinaemia (>0.4 mg/ ml) or hypertriglyceridaemia (>10 g/l) since this can interfere with measurements. If subjects with unknown status on these points are included and have PCT measurements, the measuring-equipment will detect these conditions.
5. Subjects suffering from a blood disorder, where daily sampling of 7 ml of blood for maximally 28 days (210 ml distributed on 28 days) will be an inconvenience or a potential risk, which could compromise the safety of the subject.

3.3 Treatment During Trial

The aim of the PCT guided treatment is to reduce time to relevant treatment of a serious infection and thereby to reduce the mortality. All subjects will receive the standard-of-care evaluations and therapeutic interventions recommended in the ICU at which the patient is admitted to. Subjects in the PCT measurement group will additionally receive expanded diagnostics and treatment should the PCT levels be found to high and/or increasing (see section 3.3.1 for definitions).

Access to results of PCT measurements of any kind (semi-quantitative or quantitative) at any time in the study period is not allowed for patients randomised to the control arm.

The PASS study group in collaboration with the PASS Steering Committee, will issue guidelines for the composition of the interventions that a high or increasing PCT level would mandate. Some variation between sites is acceptable, whereas all patients within a given ICU should follow that ICU's guidelines. The guidelines will be updated when new information becomes available. In the guidelines, there may be several alternatives indicated for a given situation. The investigator is not mandated to follow the guidelines.

3.3.1 Procalcitonin levels and diagnostic and therapeutic consequenses

The situation mandating additional interventions in the the PCT intervention arm is based on the following criteria:

- PCT levels ≥ 1.00 ng/ml

and

- The PCT level increases one day to the next or has an irrelevant decrease of < 10%

The daily assessment of PCT guided interventions will be as follows:

- Subjects with PCT levels ≥ 1.00 ng/ml based on the first determination after enrolment into the study will follow the principles for interventions as detailed below.
- Subjects with PCT levels ≥ 1.00 ng/ml and with a day (n) to day (n+1) PCT increase or a decrease of < 10% (irrelevant decrease) will follow the principles for interventions as detailed below.
    - Microbiology: blood cultures, airway cultures, urine cultures and samples from any other suspected foci.
    - Considerations of whether to perform diagnostic imaging: one or more of the following: Chest X-ray, Ultra-sonic examination of suspected focus, Computerised Tomography of relevant areas, Magnetic Resonance imaging of relevant areas, other imaging techniques.
    - Surgical drainage of possible un-drained foci
    - Antimicrobial therapy expansion. Treatment will be guided by any relevant findings: microbial or diagnostic imaging, or other findings. If focus and micro organism of infection is not clear steps will be:

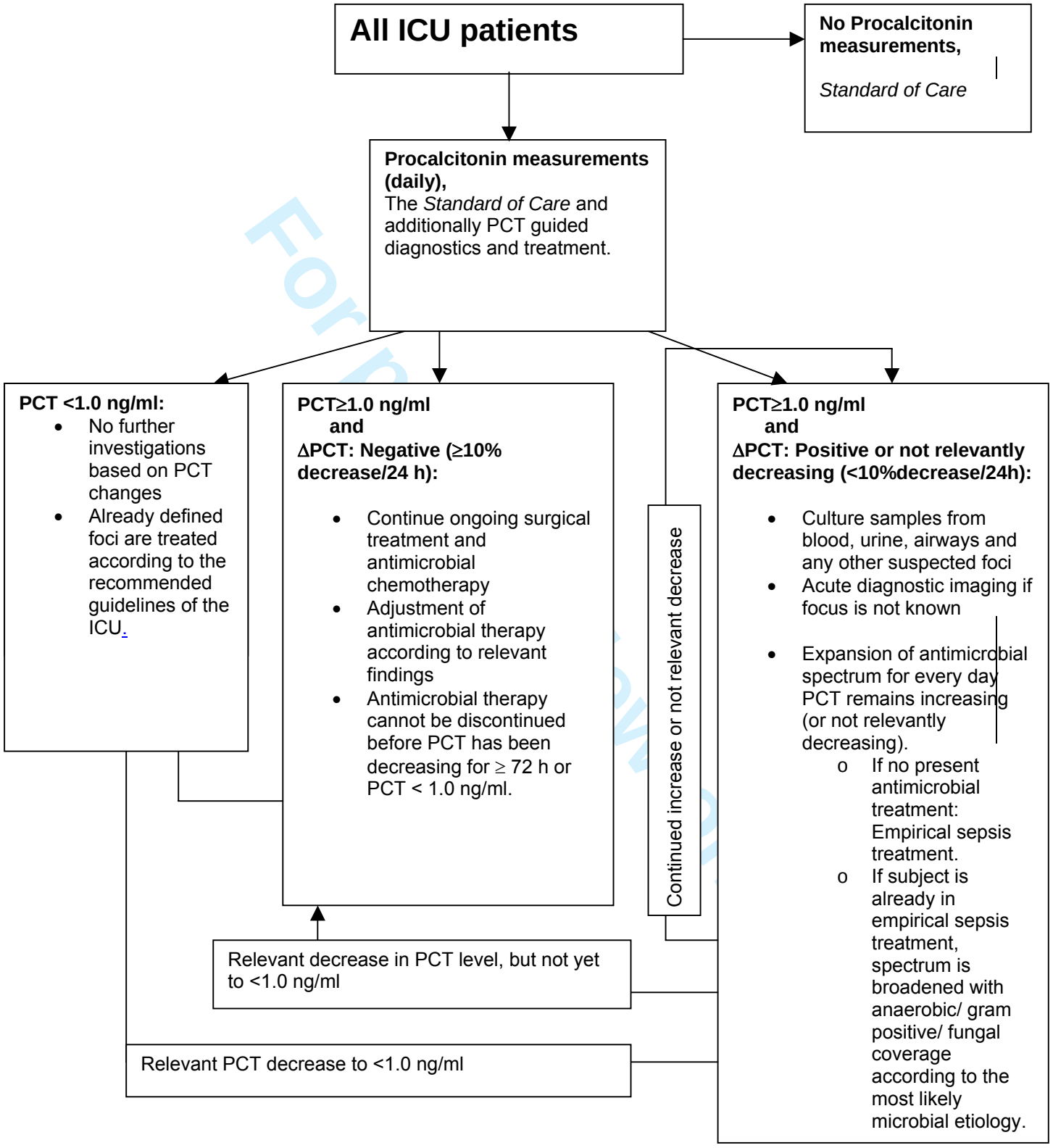
        1) Empirical sepsis treatment

        2) Empirical sepsis treatment with anaerobic and gram positive coverage

        3) Empirical sepsis treatment with anaerobic and gram positive coverage and/ or fungal treatment

- Subjects with PCT levels < 1.00 ng/ml will continue to receive standard-of-care
- Subjects with PCT levels ≥ 1.00 ng/ml and with a day-to-day PCT **decrease** of ≥ 10% will continue to receive standard-of-care.

Precise guidelines for this (antimicrobial) treatment will be made specifically for every ICU in concordance with the local choices regarding antimicrobial agents. For PCT guided diagnostics and treatment algorithm, see Diagram 1:



- **Antimicrobial treatment is NOT to be discontinued if PCT is increasing and > 1.0 ng/ml**
- **When treatment of infection is relevant, PCT normally decreases in less than 18 h. If PCT is still not decreasing at the next-coming measurement after a therapy shift, a new (expanded) strategy is to be instituted**

3.3.2 Change of PCT-guidance strategy during the trial

3.3.2.1 Randomised PCT-guided interventions

Subjects may **discontinue** the interventions initiated on the basis of PCT measurements only in case the benefit: risk ratio for these interventions is not acceptable to the treating physician. The specific concern will be collected.

3.3.2.2 The non-PCT guided interventions

The recommended interventions based on other information than PCT measurements should always be instituted and continued when relevant from a clinical judgement.

3.3.3 Antimicrobial Drugs and Dosages

All antimicrobial drugs prescribed on basis of an increasing PCT must be prescribed by the investigator or an intensive care physician, who has been sufficiently instructed in all aspects of the trial. The investigator must check for possible drug-drug interactions between any of the drugs prescribed guided by PCT changes and other agents that may be metabolised via the same enzyme systems or organs. To assist the investigator, information on this topic is included in the Manual of Operational Procedures. Also, the product label of each drug prescribed should be reviewed.

General principles that will be followed regarding antimicrobial therapy of sepsis are:

- Antimicrobial agents are prescribed, when possible, according to the resistance pattern of the causative microorganism.
- When the causative microorganism is not known, antimicrobial agents are prescribed according to knowledge of which microorganisms normally and possibly infect the suspected focus.
- When neither the microorganism nor the focus of infection is known, one or more broad spectrum antimicrobial agents are selected. If the effect is not sufficient, the spectrum of the used antimicrobial agents is additionally expanded, often with anaerobic active agents, gram positive active agents and antifungal agents. Conversely, if the effect is sufficient, the spectrum of used antimicrobial agents is narrowed according to knowledge of focus and causative microorganism.
- In empiric sepsis treatment, a combination of a ß-lactam/ Carbapenem + a fluor-quinolone is chosen if not contra indicated in the specific subject. This treatment can be

supplemented with nitroimidazoles, glycopeptides, oxazolidinones and azoles. More specific treatment regimes are initiated and guided by findings regarding the causative microorganism and/or focus of infection.

Dosages of antibiotics are decided according to the recommendations of the specific ICU.

The toxicity management guidelines detailed below refer to all components of the antimicrobial treatment used in the trial.

3.3.3.1 Overdose and Toxicity

Antimicrobial agents may be interrupted because of the development of adverse events (AEs, see section 6.1 for definitions) at the discretion of the investigator and according to the severity of the AE. The dose of all antimicrobial drugs may be reduced, interrupted or reintroduced according to standard practice at the time, and depending on the severity of the AE.

Subjects who require a dose modification should be re-evaluated on a daily basis.

The investigator is responsible for taking appropriate precautions to ensure that the risk of developing toxicity is minimised, that the subject is monitored for the development of toxicity, and if such toxicities do occur, take appropriate action to minimise their effects.

4 MEASUREMENTS AND EVALUATION

4.1 Time and Events Schedule

A flow chart showing the timing of trial procedures (Clinical and Laboratory) is shown in Table 1.

An initial pre-entry (screening) assessment for eligibility will be performed as soon as possible after the patient is admitted to the ICU. The patient should be randomised no later than 24 hours after the time of admission. Evaluations will then be carried out at entry (Day 1), and thereafter daily as long as the patients remains in the ICU. After discharge, the course of disease is collected in less detail and the survival status determined day 28, 60, 90 and 180 after enrolment in the trial.

4.1.1 Pre-entry Evaluations

The site must obtain subject consent in the form of a written informed consent form prior to the initiation of **any** pre-entry procedures as outlined in this protocol. The consent form must be approved by the IEC of each participating site.

The pre-entry evaluation will be conducted the first day of the trial by an investigator in the ICU and will include an evaluation of whether the patient fulfils the requirements for enrolment in this trial (see section 3.2.2 and 3.2.3.

Subjects who fail to meet the entry criteria may not be re-screened for this protocol until 28 days after the failed pre-entry evaluation. Hence, enrolment of such patients will require that the patient is re-admitted to the ICU after at least 7 days outside of the ICU after the time of the first screening.

4.1.2 Baseline (Day 1) Evaluations

The following evaluations should be performed at baseline (Day 1):

Note: For this trial, Baseline (Day 1) is defined as the day on which the subject has his/her first blood sample for PCT measurement. The following data are to be collected on day 1:

- Demography including date of birth, weight, height, and indication for admittance to the ICU
- Infections found in the subject in this hospital admission prior to admittance to the ICU.
- Present infection focus/ etiologic microorganism

- APACHE II score (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale)

- Current medical conditions

- Pre-admittance daily function and health state:

Professional career:	1) Student, 2) Part time work, 3) Full time work, 4) Early retirement, 5) Retired
Health:	1) Congenital handicapped, 2) Acquired handicap, 3) Chronic disabling disease, 4) Chronic non-disabling disease, 5) Healthy
Self-supportance:	1) Lives in nursing home, 2) Lives in a flat connected to a nursing home, 3) Own home with external help ≥ once / day, 4) Own home with external help < once daily, 5) Own home, no help required
Hospital need:	1) ≥ 3 months admitted to a hospital/ last year, 2) 1-3 months admitted to a hospital/ last year 3) 1-30 days admitted/ last year, 4) No admissions, ambulatory visits ≥ 6/ last year, 5) No admissions, ambulatory visits 1-5/ last year, 6) No admissions, No ambulatory visits/ last year

- Adverse events/ other complications to treatment given in this hospital admission (ongoing clinical conditions at Day 1 shall be recorded in the “Adverse Event and Medical Condition Form” of the CRF at this time, regardless of the fact that such conditions may not subsequently be found to fulfil the definitions for an adverse event (see section 6.1))

- Haematology: *haemoglobin, platelet count (WBC count mentioned as part of APACHE II)*

- Clinical chemistry: Albumin, Bilirubin, Factor 2-7-9, Alanin Amino Transferase (ALAT)/ Aspartate Amino Transferase (ASAT), Alcaline Phosphatase, Creatinine, Carbamide, Na^+, K^+, Phosphate, Ca^{2+}, C-reactive protein (some are also mentioned as part of APACHE II).

- Baseline PCT

The daily PCT determination is done real-time at the Department of Clinical Biochemical Department, Hvidovre Hospital, using the EC-approved measuring instruments and reagents. For each subject, the same methodology should be used throughout the trial period. The KRYPTOR® PCT BRAHMS sensitive assay is the accepted standard assay. Other licensed assays may be used instead if judged by the PASS steering committee to have a comparable performance compared to the indicated assay.

4.2 On Trial Evaluations

On trial assessments will be completed at the following time-points unless otherwise specified:

While admitted to the ICU, the following information will be registered unless specified otherwise:

Daily while patient is admitted to the ICU:

- Clinical signs of new (nosocomial) infections
- Microbiological or radiological evidence of new (nosocomial) infection
- Defined Day Doses of antimicrobial chemotherapy
- APACHE II score (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale)
- Occurrence of sepsis, severe sepsis, septic shock, DIC. Assessment of Glasgow Coma Scale, measurement of Blood Pressure (systolic blood pressure < 90), days with artificial ventilation, Factor 2-7-9 < 0.7, creatinine (increase factor 3 from baseline), MODS.
- Adverse events/ other complications to treatment given in the ICU (ongoing clinical conditions at Day 1 shall be recorded in the “Adverse Event and Medical Condition Form” of the CRF at this time, regardless of the fact that such conditions may not subsequently be found to fulfil the definitions for an adverse event (see section 6.1))
- Haematology: haemoglobin, platelet count WBC (WBC count also mentioned as part of APACHE II)
- Clinical chemistry: Albumin, Bilirubin, Factor 2-7-9, Alanin Amino Transferase (ALAT)/ Aspartate Amino Transferase (ASAT), Alcaline Phosphatase, Creatinine, Carbamide, Na^+, K^+, Phosphate, Ca^{2+}, C-reactive protein (some are also mentioned as part of APACHE II).

- Blood sample for PCT determination
- Diagnostic imaging procedures performed
- Non-routine microbiological sample taken
- Surgical procedures performed
- Change in antimicrobial chemotherapy

At the day of discharge from ICU or day of death or later:

- Mortality and time of death, and the cause hereof
- $AUC_{Procalcitonin}$ (at discharge from the ICU) (will remain blinded in the control arm)
- Discharge and post-discharge daily function and health state (obtained on day 30 and 180):

Professional career:	1) Student, 2) Part time work, 3) Full time work, 4) Early retirement, 5) Retired
Health:	1) Congenital handicapped, 2) Acquired handicap, 3) Chronic disabling disease, 4) Chronic non-disabling disease, 5) Healthy
Self-supportance:	1) Lives in nursing home, 2) Lives in a flat connected to a nursing home, 3) Own home with external help ≥ once / day, 4) Own home with external help < once daily, 5) Own home, no help required.
Hospital need:	1) ≥ 3 months admitted to a hospital/ last year, 2) 1-3 months admitted to a hospital/ last year 3) 1-30 days admitted/ last year, 4) No admissions, ambulatory visits ≥ 6/ last year, 5) No admissions, ambulatory visits 1-5/ last year, 6) No admissions, No ambulatory visits/ last year

After discharge from ICU while patient is still admitted to hospital

- Clinical signs of new (nosocomial) infections

- Microbiological or radiological evidence of new (nosocomial) infection
- Defined Day Doses of antimicrobial chemotherapy
- Current medical conditions (including acute organ failures)
- Diagnostic imaging procedures performed
- Surgical procedures performed
- Blood sample for PCT determination – done daily

4.3 Trial drugs

Drugs prescribed on basis of PCT levels and changes belong to following categories: Antibacterial chemotherapeutics and Antifungal chemotherapeutics. Drugs from these categories will also be prescribed for the control group (and in patients not included in the trial), when indicated from other findings than level/change of PCT. An exhaustive list of drugs, used in the participating ICU´s (and thereby also in the trial subjects and controls) is given in appendix

4.3.1 Dosing Details

The following details on dosing of all prescribed antimicrobials during the study period must be recorded in the “Medication form” in the CRF.

- Date of initial therapy
- Dose at each dosing change, together with reason for change
- Date of last dose of each agent
- Reason for discontinuation
- Date of resumption of therapy

4.3.2 Collection of Blood Samples for Daily Analysis

Plasma from the PCT group and the control group will be collected early each morning (01.00 a.m.-06.00 a.m.) and will be transported to the Department of Clinical Microbiology Hvidovre Hospital, DK-2650 Hvidovre (or other laboratories, that can provide a PCT analysis real-time and with an analysing method which is approved by the PASS coordinating centre) and analysed immediately hereafter. The results from this analysis will be communicated via a

webbased cryptized licensed answering system every day to the Intensive Care Units for patients randomised to the PCT intervention arm or concealed for patients randomised to the control arm. Remaining material for the blood samples will hereafter be frozen for later analysis of other biochemical, biological and genetic markers (-80°C). Once the trial has been completed, the coupling of these samples to person-identifiers will be broken, and hence subsequent analyses done without any possibility to connect the results to individual persons involved in the trial. For detailed instructions regarding the collection, labelling, processing and transport of samples, see the Manual of Operational Procedures.

It is the responsibility of the investigator (to be assisted by the courier service and PASS coordinating office) to ensure that all trial samples for transport are appropriately handled, packed and transported.

4.3.3 Genetic markers (PASS-sub-study)

The PASS-sub-study has three aims: 1. quality assessment of the procalcitonin analyzes used in the PASS-Study, 2. to investigate the relation between levels of procalcitonin and other biomarkers and 3. to investigate if genetic markers can be used to gain an early knowledge of the course of critical illness.

To investigate this, we will use the remaining material from the blood samples collected for the PASS-Study. Blood plasma and DNA material will be frozen at minus 80 degrees Celcius. The PASS-Sub-study, therefore, will not mean any inconvenience for the study subjects and no additional blood sampling. This material will be kept in anonymous form for 5 years after the closure of the PASS-Study. Known hereditary diseases will not be examined.

Regarding 1.: In a randomly assigned set of blood samples, and additionally in samples that have shown extreme PCT values a double determination will be performed to assess the inter-assay variability.

Regarding 2.: Other biomarkers as interleukin-6 and soluble TNF-α receptor have been, and are still under assessment as predictive markers at sepsis and in other infectious diseases. In plasma, these and other markers will be analyzed after the closure of the PASS-Study to assess the value of these markers compared to PCT, also as prognostic markers.

Regarding 3.: Genetic polymorphisms (e.g. mannan-binding lectins, interleukins, complement, immunglobulin receptor, Toll-like receptor 1-9, and Factor V Leiden) are related to the prognosis at sepsis and can, to some degree, identify patient groups with a high risk of a fatal course of

the disease. An increasing number of international studies have during the latest years investigated the relation between the genetic disposition of patients and the course of infectious diseases, but often, these studies have been small and without sufficient statistical power to conclude on these issues.
The statistical power in investigating the relation between genetic polymorphisms and mortality in sepsis depends on the frequency of a certain allele, the mortality in the study population and the size of the population.

Directly applied on the study population of the PASS-Study with 1000 cases of sepsis (mortality ~25%) it will result in a 80 % statistical power to show a 2-fold increase in mortality for an allele that is found in 3% of the population. For alleles that are more frequent, we will be able to show less than a 2-fold increase in mortality. As an example of this, the homozygote forms of TNF-α, IL-1β, and PAI-1 have a frequency of 5, 7, and 14%, respectively. Heterozygote forms of TLR4 and factor V Leiden have a frequency of 9 and 7%.

5 DATA ANALYSIS METHODS

5.1 Sample Size Determination

The trial will randomise (1:1) 1,000 subjects into two treatment arms:

1: Control arm

2: The PCT guided intervention arm

With a sample size of 500 per group and an assumed mortality rate of 25% in the control group and 17.5 % in the PCT group there will be 80% probability that a negative result (Confirming the Null Hypothesis) is true. At the same time there will be < 5% probability of falsely declaring the alternative hypothesis correct. [Power 80%, stringency 5%]. Sample Size calculations via Dept. of Statistics, UCLA, California, USA.

5.2 General Considerations

5.2.1 Analysis Populations

The primary population for analyses of the efficacy and safety data will be the intention to treat population, including all randomised subjects who have at least one blood sample made for PCT measurements.

Response to PCT guided diagnostic and therapeutic interventions will also be investigated descriptively by summary statistics for various sub-groups, e.g. gender, other demographic variables, Baseline APACHE II score, and pre-admittance health assessment.

5.2.2 Interim Analysis

Safety and efficacy data will be reviewed when 250, 500 and 750 subjects have completed the trial period (until discharge from the hospital or death, maximally 28 days), or at least every 6 th month, and assessments will be made by an independent Data and Safety Monitoring Board (DSMB). A cut-off date will be specified at this point and all treatment failure and adverse event data before this date will be used.

The Peto method of repeated significance testing will be used to test for treatment difference and a p-value of 0.001 will be used as the significance level at the interim analysis, giving a significance level of 0.05 for the final analysis once all patients have completed the trial.

Stopping the trial will not be based purely on a statistical decision but also on the recommendation of the DSMB.

5.2.3 Other Issues

All subjects will remain in the trial and be followed-up until day 180.

5.3 Efficacy

5.3.1 Primary Efficacy Endpoint

The primary efficacy analysis will be the comparison of the two treatment groups with respect to the incidence of mortality within 28 days after enrolment in the trial. Mortality is defined as all-cause mortality. Subjects not followed for the entire duration of the trial (i.e. lost to follow-up) will be counted as survivors. Very few patients will be lost to follow up for the primary endpoint, because of the Danish Central Person Register (CPR), where all deaths in Denmark are registered. Only subjects who permanently move their address to another country within 30 days after ICU admission can be lost to follow-up. The stratified log-rank test and Kaplan Meier estimates will be used.

5.3.2 Secondary Efficacy Endpoint(s)

5.3.2.1 Other mortality assessments

The proportion of subjects, who survive to different points of time (at discharge, after 60, 90 and 180 days, counting after ICU admission). The log rank test and Kaplan-Meier estimates will be used. Differences in proportions of survivors will be assessed using the Mantel-Haenzel Chi Square test and Wilcoxon test. Subjects with missing mortality data will be classified as survivors.

5.3.2.2 Other parameters than mortality

- Defined day doses of antimicrobial therapy in each arm
- Occurrence of sepsis, severe sepsis, septic shock, DIC. Assessment of Glasgow Coma Scale, measurement of Blood Pressure (systolic blood pressure < 90), days with artificial ventilation, Factor 2-7-9 < 0.7, creatinine (increase factor 3 from baseline), MODS.
- SOFA score daily (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale).
- $AUC_{Procalcitonin}$ for the Procalcitonin-measuring group and for the control group.
- Number of diagnostic images after admission to the ICU.
- Number of non-routine microbiological sample taken after admittance to the ICU.
- Number of surgical procedures during the trial
- Time to the first change in antimicrobial chemotherapy after admittance to the ICU
- Occurrence of new clinically, microbiologically or radiologically diagnosed infections while admitted to the ICU
- Discharge and post-discharge daily function and health state

For endpoints that have normally distributed numbers, t-test will be used in assessment of statistical significance. If not normally distributed, Mantel-Haenzel Chi Square test and the Wilcoxon test, will be used.

Exploratory analysis of adjustments for possible confounders present at baseline for the analysis presented above will be performed using Cox proportional hazards and Logistic regression modelling (as appropriate).

5.3.3 Combined evaluation of mortality / occurrence of serious bacterial infection while admitted to the ICU

The proportion of patients who die during the trial period or who experience occurrence of a serious bacterial infection (sepsis, severe sepsis, septic shock, Disseminated Intravascular Coagulation (DIC) or Multi Organ Dysfunction Syndrome (MODS) (which ever came first) as a function of time since trial initiation. In this analysis, patients discontinuing the randomised treatment for other reasons before having failed in this analysis will be censored from the time of discontinuation.

5.4 Safety

Adverse events will be tabulated by treatment group, maximum intensity, attributability to various antimicrobial agents and by seriousness. Treatment related adverse events that lead the subject to prematurely discontinue one or more of the originally prescribed antimicrobial agents will also be summarised.

Clinical chemistry and haematology results will be presented by summary statistics and quartile plots of measured results. Change from baseline for these results will also be presented. Baseline is defined as the laboratory data collected at Day 1 (before the first blood sample for PCT analysis). Subjects must have both a baseline and an “on treatment” measurement to be included in the change from baseline analysis.

Treatment emergent toxicity grades will be presented for each graded laboratory parameter by treatment group. A graded toxicity is considered treatment emergent if it develops or increases in intensity, post Day 1. Treatments will include established and approved antimicrobial treatments, which are already used daily in the participating ICU´s.

Concurrent medications and blood products will be summarised by randomised treatment group.

6 ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

As mentioned other places in this protocol, the direct inconvenience for subjects in this study is sampling of 7 ml of whole blood daily in the same session as the routine blood samples are made, every morning. Therefore it is reasonable to expect that AE´s and SAE´s as a direct consequence of this blood sampling will not occur. Indirect AE´s as a consequence of potential overly treatment are likewise not likely to occur according to the available literature on the issue, especially because the most striking result of the previously published RCT´s is a reduction of antibiotic exposure in the PCT-guided group.
All interventions, that are performed in this study are well-known, thoroughly tested and accepted treatments, so it does not seem reasonable to apply the same procedures for this study regarding AE´s as e.g. a study where a new drug is to be assessed for safety (or effect)

Investigators will, however, have the opportunity to report events, that they fing unexpected in the Case Report Form. In this part of the CRF, it is possible to classify unexpected events in groups of "relatedness" to the antimicrobial treatment as "no relation", "unlikely relation", "possibly related", "probably related" or "definitely related.

Serious unexpected events or unexpected events

Serious inexpected events and unexpected events, that can be related to the antimicrobial treatment will in both treatment groups be reported to the Danish Medicines Agency "Lægemiddelstyrelsen" according to the Danish legislation on this point

The primary and the secondary endpoints that are registered daily in the case report form are all *adverse events or serious adverse events, i.e. death, complications to sepsis, increased antibiotic exposition and prolonged hospital stay. These are registered routinely and daily in the part of the CRF dealing with effects of the treatments. All patients are at inclusion in the study threatened by potentially lethal illnesses.*

7 TRIAL ADMINISTRATION

7.1 Data Collection

Case Report Forms (CRF) will be provided for each subject by the PASS coordinating centre. All data on the CRFs must be entered legibly in black ink or typed, in Danish or English. Amendments and errors on the CRFs should not be erased, covered with correction fluid or completely crossed-out; rather, a single line should be drawn through the error and the correction initialled and dated by the investigator, authorised colleague or co-worker. An explanatory note for the change should also be written on the CRF. Any requested information which is not obtained or unanswerable should be identified by entering 'ND' (not done). An explanation must be documented for any missing data. CRFs must be completed regularly and should never bear the participant's name. Participants will be identified by initials, date of birth and subject trial number only.

The investigator (or a person appointed by the investigator) must sign and date a declaration on the CRF attesting to his/her responsibility for the quality of all data recorded and that the data represents a complete and accurate record of each subject's participation in the trial.

Details and procedures for the completion of the CRFs are specified in the Manual of Operational Procedures.

All trial CRFs will be plain paper copies – the original being the investigators copy. After completion of each page of the CRF, the investigator will send it by fax to the PASS coordinating centre. Pages will be reviewed and clarified in accordance with the protocol specific Review and Validation Manual. The data will be double entered (punched and verified) by separate data entry specialists to produce data files.

Identical validation checks will be performed on each database. Data failing any check will be flagged for output on a Data Clarification Report (DCR) and sent to the relevant investigator for resolution. In such cases the investigator is requested to sign and date any explanation or correction. On return, the database will be updated appropriately and the original DCR stored with the original CRF.

The database(s) will be subject to agreed Quality Control (QC) checks before authorisation. The data will be subsequently analysed according to the methods outlined in Section 5.

7.2 Regulatory and Ethical Considerations

7.2.1 Regulatory Authority Approval

The co-ordinator (in collaboration with the PASS coordinating centre) will obtain approval from the appropriate regulatory agency prior to initiating the trial at a site.

This trial will be conducted in accordance with ICH-GCP and all applicable regulations, including, where applicable, the Declaration of Helsinki, June 1964, as modified by 52nd WMA General Assembly, Edinburgh, Scotland, October 2000 (see Appendix 1).

7.2.2 Ethics Approval

It is the investigator's responsibility to ensure that this protocol is reviewed and approved by the appropriate local Independent Ethics Committee (IEC). The IEC must also review and approve the site's informed consent form (ICF) and any other written information provided to the subject prior to any enrolment of subjects, and any advertisement that will be used for subject recruitment. The co-ordinator and/or the investigator must forward to the PASS coordinating centre copies of the IEC approval and the approved informed consent materials, which must be received by the PASS coordinating centre prior to the start of the trial.

If, during the trial, it is necessary to amend either the protocol or the informed consent form, the co-ordinator and/or investigator will be responsible for ensuring the IEC reviews and approves these amended documents. IEC approval of the amended ICF must be obtained before new subjects consent to take part in the trial using this version of the form. Copies of the IEC approval of the amended ICF and the approved amended ICF must be forwarded to the PASS coordinating centre as soon as available.

7.2.3 Subject Informed Consent

The investigator or his/her designee will inform the subject of all aspects pertaining to the subject's participation in the trial.

The process for obtaining subject informed consent will be in accordance with all applicable regulatory requirements. The investigator or his/her designee and the subject/ witness of an oral informed consent/ subjects legally acceptable representative must both sign and date the ICF before the subject can participate in the trial. Following types of informed consent can be accepted because of the nature of the ICU setting and the physical and/ or mental state of the subjects.

1)Ability to understand and provide written informed consent to participate in this trial,

or

2)Ability to understand and provide oral informed consent in presence of at least one impartial witness who should sign and personally date the consent form

or

3)The subjects legally acceptable representative can understand and provide written informed consent if the subject is not capable of this because of the present mental or physical condition of the subject.

The subject will receive a copy of the signed and dated form and the original will be retained in the site trial records. The decision regarding subject participation in the trial, that is made by the subject, is entirely voluntary. The investigator or his/her designee must emphasize to the subject that consent regarding trial participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

If the ICF is amended during the trial, the investigator must follow all applicable regulatory requirements pertaining to approval of the amended ICF by the IEC and use of the amended form (including for ongoing subjects).

7.3 Trial Monitoring

In accordance with applicable regulations, good clinical practice (GCP), monitors will periodically contact the site, including conducting on-site visits. The extent, nature and frequency of on-site visits will be based on enrolment rate, the quality of the documents provided by the site, consistency of follow-up of the patients according to this protocol.

During these contacts, the monitor will:

- check and assess the progress of the trial

- review trial data collected
- conduct Source Document Verification
- identify any issues and address their resolution

This will be done in order to verify that the:

- data are authentic, accurate, and complete
- safety and rights of subjects are being protected
- trial is conducted in accordance with the currently approved protocol (and any amendments), GCP, and all applicable regulatory requirements

The investigator agrees to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and any relevant issues.

In addition to contacts during the trial, the monitor will also contact the site prior to the start of the trial to discuss the protocol and data collection procedures with site personnel.

At trial closure, monitors will also conduct all activities as indicated in Section 7.5, Trial and Site Closure.

7.4 Quality Assurance

At its discretion, the PASS coordinating centre may conduct a quality assurance audit of this trial. If such an audit occurs, the investigator agrees to allow the auditor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor to discuss findings and any relevant issues. A guideline for audit is available at the PASS coordinating centre.

In addition, regulatory agencies may conduct a regulatory inspection of this trial. If such an inspection occurs, the investigator agrees to allow the inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the inspector to discuss findings and any relevant issues.

7.5 Trial and Site Closure

Upon completion of the trial, the following activities, when applicable, must be conducted by the monitor in conjunction with the investigator, as appropriate:

- return of all trial data to the PASS coordinating centre

- data clarifications and/or resolutions
- review of site trial records for completeness
- shipment of stored samples to assay laboratory

In addition, the steering committee reserves the right to temporarily suspend or prematurely discontinue this trial either at a single site or at all sites at any time and for any reason. If such action is taken, selected members of the PASS steering committee and/or the PASS coordinating centre will discuss this with the Investigator (including the reasons for taking such action) at that time. The PASS coordinating centre will promptly inform all other investigators conducting the trial if the trial is suspended or terminated for safety reasons. The investigators will inform their local/regional/national regulatory authorities (as appropriate) of the suspension or termination of the trial and the reason(s) for the action. If required by applicable regulations, the investigator must inform the IEC promptly and provide the reason for the suspension or termination.

If the trial is prematurely discontinued, all trial data must be returned to the PASS coordinating centre.

7.6 Records Retention

In accordance with applicable regulatory requirements, following closure of the trial, the investigator will maintain a copy of all site trial records in a safe and secure location. The PASS coordinating centre will inform the investigator of the time period for retaining these records in order to comply with applicable regulatory requirements.

7.7 Information Disclosure and Inventions

7.7.1 Confidentiality

The investigator and other trial site personnel will keep confidential any information provided by the co-ordinating centre (including this protocol) related to this trial and all data and records generated in the course of conducting the trial, and will not use the information, data, or records for any purpose other than conducting the trial. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the investigator or trial site personnel; (2) information which it is necessary to disclose in confidence to an IEC solely for the evaluation of the trial; or (3) information which it is necessary to disclose in order to provide appropriate medical care to a trial subject.

7.7.2 Publication

The findings from this trial is intended to be published in peer-reviewed journals. The steering committee decides whether abstracts are to be submitted to conferences, and how the results are distributed if more than one manuscript is to be drafted.

Authorship: The trial group as a whole will appear in an appendix in all published manuscripts. Co-authors are selected after a fair evaluation of primarily number of patients entered in to the trial and the level of involvement in the drafting of the manuscript. Providing that several manuscripts are to be drafted, a fair rotation among the participating clinical sites of co-authorship slots will be done taking in to consideration the number of patients enrolled.

7.8 Indemnification and Compensation for Injury

The insurance that covers liability in relation to patient care in Denmark, *Patientforsikringen* will cover all liability aspects of the conduct of this trial[45-46].

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Table 1: Clinical and laboratory Evaluations

Evaluation	Day (screening & baseline)		Day (counting after admission to ICU) (follow-up)				
	1	Day=Dis-charge/ death	28	30	60	90	180
Informed Consent	X						
Entry Criteria	X						
Demography	X						
APACHE II	X	X					
Infections during this hospital admission	X						
Current medical conditions	X	X					
State of daily function and health	X			X			X
Mortality		(X)	X		X	X	X
Baseline PCT	X						
$AUC_{procalcitonin}$		X					
Concurrent Medications[a]	X	X		X	X	X	X
Haematology	X	X					
Clinical chemistry	X	X					
Adverse events	X[a]	X					
Serious Adverse Events	X[a]	X		X	X	X	X

a Adverse events and serious adverse events are registered daily

9. APPENDICES

Appendix 1

Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.
9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.
19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
20. The subjects must be volunteers and informed participants in the research project.
21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.
23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain

informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Appendix 2: Abbreviations

AE	**Adverse Event (AE)**
ALAT	**Alanine Aminotransferase (SGOT)**
APACHE II	**Acute Physiology And Chronic Health Evaluation II**
ASAT	**Aspartate Aminotransferase (SGPT)**
CDC	**Centers for Disease Control**
CRF	**Case Report Form**
DDD	**Defined Day Doses**
DIC	**Disseminated Intravascular Coagulation**
DSMB	**Data Safety Monitoring Board**
ICU	**Intensive Care Unit**
IEC	**Independent Ethics Committee**
IL-6	**Interleukin 6**
MODS	**Multi Organ Dysfunction Syndrome**
PASS	**Procalcitonin and Surivival Study**
PCT	**Procalcitonin**
SAE	**Serious Adverse Event**
TNFα	**Tumor Necrosis Factor α**
WBC	**White Blood cell Count**

Appendix 3: Table of conversion factors for laboratory units

TEST	CONVENTIONAL		SI	
	Unit	Factor	Unit	Factor
Haemoglobin	g/dl	0,6206	mmol/l	1,61
Platelets	Thou/mm^3	0,001	[a]x10^9/l	1000
Hyponatraemia (↓ Sodium)	mEq/l	1,0	mmol/l	1,0
Hypernatraemia (↑ Sodium)	mEq/l	1,0	mmol/l	1,0
Hypokalaemia (↓ Potassium)	mEq/l	1,0	mmol/l	1,0
Hyperkalaemia (↑ Potassium)	mEq/l	1,0	mmol/l	1,0
Hypoglycaemia (↓ Glucose)	mg/dl	0,0555	mmol/l	18,0
Hyperglycaemia (↑ Glucose)	mg/dl	0,0555	mmol/l	18,0
Hypocalcaemia (↓ Calcium)	mg/dl	0,2495	mmol/l	4,0
Hypercalcaemia (↑ Calcium)	mg/dl	0,2495	mmol/l	4,0

[a] No SI unit

For example: Haemoglobin 9,5 g/dl - multiply by factor 0,6206 → 5,9 mmol/l

Appendix 4: Table with the used antibacterial and antifungal drugs used in the 6 participating Intensive Care Units.

Generic name	Comercial name (s)
Benzyl-Penicillin	Penicillin"Leo", Penicillin"Rosco" Benzyl-Penicillin"Panpharma"
Phenoxymethyl-Penicillin	Calcipen ®, Pancillin ®, Primcillin ®, Rocilin ®, Vepicombin ®"DAK"
Dicloxacillin	Dicillin ®, Diclocil ®
Flucloxacillin	Heracillin
Amoxicillin	Amoxicillin"NM", Flemoxin Solutab ®, Imacillin ®, Imadrax ®,
Amoxicillin+Clavulanic Acid	Bioclavid, Bioclavid Forte, Spektramox ®
Ampicillin	Ampicillin"Vepidan", Doktacillin, Pentrexyl ®
Piperacillin	Ivacin ®, Pipril
Piperacillin+Tazobactam	Tazocin ®
Pivampicillin	Pondocillin ®
Pivmecillinam/ Mecillinam	Selexid ®
Cefalexin	Keflex ®
Cefalotin	Keflin ®
Cefepim	Maxipime ®
Cefotaxim	Claforan ®
Ceftazidim	Fortum ®
Ceftriaxon	Rocephalin ®
Cefuroxim	Zinacef, Cefuroxim Stragen, Zinnat ®
Aztreonam	Azactam ®
Meropenem	Meronem ®
Imipenem+cilastatin	Tienam ®
Azithromycin	Zitromax ®
Clarithromycin	Klacid ®, Klacid ® Uno, Klaricid, Zeclar
Erythromycin	Abboticin ®, Abboticin ® Novum, Erycin ®, Escumycin, Hexabotin ®
Roxithromycin	Surlid ®, Forimycin ®, Roximstad, Roxithromycin"Copyfarm", Roxithromycin"UNP"
Doxycyclin	Vibradox ®
Lymecyclin	Tetralysal ®
Oxytetracyclin	Oxytetral ®
Tetracyclin	Tetracyclin"AL", Tetracyclin"DAK", Tetracyclin"SAD"

Gentamicin	**Garamycin ®, Gentacoll ®, Hexamycin, Septopal, Septopal Mini**
Netilmicin	**Netilyn**
Tobramycin	**Nebcina ®, Tobi ®**
Moxifloxacin	**Avelox**
Ciprofloxacin	**Ciproxin ®, Cifin, Ciprofloxacin"1A Farma", Ciprofloxacin"2K Pharma", Ciprofloxacin"Alpharma", Ciprofloxacin"Biochemie", Ciprofloxacin"Gea", Ciprofloxacin"Ratiopharm", Sancipro, Sibunar ®**
Ofloxacin	**Tarivid ®**
Norfloxacin	**Zoroxin ®**
Methenamin	**Haiprex**
Nitrofurantoin	**Nitrofurantoin"DAK", Nitrofurantoin"SAD"**
Sulfamethizol	**Lucosil ®, Sulfametizol"SAD", Sulfametizol"Ophtha"**
Trimethoprim	**Monotrim ®, Trimethoprim"1A Farma", Trimopan**
Sulfamethoxazol+Trimethoprim	**Sulfamethoxazol+Trimethoprim"SAD", Sulfotrim ®**
Clindamycin	**Dalacin ®**
Colistin	**Colimycin**
Teicoplanin	**Targocid ®**
Vancomycin	**Vancocin, Vancomycin"Abbott", Vancomycin"Alpharma"**
Fusidinsyre	**Fucidin ®**
Linezolid	**Zyvoxid ®**
Metronidazol	**Flagyl ®, Metronidazol"Alpharma", Metronidazol"DAK", Metronidazol"SAD"**
Amphotericin B	**Abelcet, AmBisome, Fungizone**
Caspofungin	**Cancidas ®**
Fluconazol	**Conasol, Diflucan ®, Fluconazol"Alpharma", Fluconazol"Copyfarm", Fluconazol"Nycomed", Fluconazol"Ratiopharm", Fluconazol"Stada", Fungal ®, Fungustatin**
Flucytosin	**Ancotil**
Ketoconazol	**Nizoral ®**
Voriconazol	**Vfend**
Ethambutol	**Myambutol ®**
Isoniacid	**Isoniacid"OBA"**
Pyrazinamid	**Pyrazinamid"Medic", Pyrazinamid"SAD"**
Rifabutin	**Rifabutin"Pharmacia"**
Rifampicin	**Rimactan ®**

Kidney failure related to broad-spectrum antibiotics in critically ill patients: secondary end point results from a 1200 patient randomized trial

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Complete List of Authors:	Jensen, Jens-Ulrik; Faculty of Health Sciences, University of Copenhagen, Copenhagen HIV Programme; University Hospital of Copenhagen, Bispebjerg, Pulmonary Medicine (L) Hein, Lars; Copenhagen University Hospital Hillerød, Department of Anesthesia and Intensive Care ; Copenhagen University Hospital Glostrup, Department of Anesthesia and Intensive Care Lundgren, Bettina; University Hospital of Copenhagen Rigshospitalet, Diagnostic Centre Bestle, Morten; Copenhagen University Hospital Hillerød, Department of Anesthesia and Intensive Care Mohr, Thomas; Copenhagen University Hospital Gentofte, Department of Anesthesia and Intensive Care Andersen, Mads; Aarhus University Hospital in Skejby, Department of Anesthesia and Intensive Care Thornberg, Klaus; Copenhagen University Hospital Gentofte, Department of Anesthesia and Intensive Care Løken, Jesper; Copenhagen University Hospital Hvidovre, Department of Anesthesia and Intensive Care Steensen, Morten; Copenhagen University Hospital Hvidovre, Department of Anesthesia and Intensive Care Fox, Zoë; Royal Free Hospital School of Medicine in London, Research Department of Infection and Population Health; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Tousi, Hamid; Copenhagen University Hospital Herlev, Department of Anesthesia and Intensive Care Søe-Jensen, Peter; Copenhagen University Hospital Herlev, Department of Anesthesia and Intensive Care Lauritsen, Anne; Copenhagen University Hospital Glostrup, Department of Anesthesia and Intensive Care Strange, Ditte; Copenhagen University Hospital Glostrup, Department of Anesthesia and Intensive Care Reiter, Nanna; University Hospital in Roskilde, Department of Anesthesia and Intensive Care Thormar, Katrin; Copenhagen University Hospital Gentofte, Department of Anesthesia and Intensive Care Fjeldbord, Paul; Aarhus University Hospital in Skejby, Department of Anesthesia and Intensive Care Larsen, Kim; Aarhus University Hospital in Aarhus, Department of Anesthesia and Intensive Care Drenck, Niels-Erik; University Hospital in Roskilde, Department of

	Anesthesia and Intensive Care Østergaard, Christian; Copenhagen University Hospital Hvidovre, Clinical Microbiology Johansen, Maria; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Nielsen, Lene; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Kjær, Jesper; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Grarup, Jesper; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Lundgren, Jens; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme; Copenhagen University Hospital Rigshospitalet, Infectious Diseases
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Keywords:	Adult intensive & critical care < ANAESTHETICS, Acute renal failure < NEPHROLOGY, Adverse events < THERAPEUTICS, Toxicity < THERAPEUTICS, Clinical trials < THERAPEUTICS

Kidney failure related to broad-spectrum antibiotics in critically ill patients: secondary end point results from a 1200 patient randomized trial

Corresponding author Jens-Ulrik Jensen, Copenhagen HIV Programme, The Panum Institute, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen N, juj@cphiv.dk

Jens Ulrik Jensen *medical doctor*[1,2], Lars Hein *anaesthetist*[3,4], Bettina Lundgren *centre director, hospital diagnostic centre* [2,5], Morten Heiberg Bestle *anaesthetist* [4], Thomas Mohr *anaesthetist*[6], Mads Holmen Andersen *anaesthetist*[7], Klaus Julius Thornberg *anaesthetist*[6], Jesper Løken *anaesthetist*[8], Morten Steensen *anaesthetist*[8], Zoe Fox *biostatistician*[1,9], Hamid Tousi *anaesthetist*[10], Peter Søe-Jensen *anaesthetist*[10,] Anne Øberg Lauritsen *anaesthetist*[3], Ditte Gry Strange *anaesthetist*[3], Nanna Reiter *anaesthetist* [11], Katrin Thormar *anaesthetist*[6], Paul Christian Fjeldborg *anaesthetist* [7], Kim Michael Larsen *anaesthetist*[12], Niels-Erik Drenck *anaesthetist*[11] Maria Egede Johansen *junior research associate*[1], Lene Ryom *junior research executive*[1], Christian Østergaard *senior research executive*[2,13], Jesper Kjær *database manager*,[1] Jesper Grarup *administrative leader* [1], Jens D. Lundgren *professor of infectious diseases*,[1,14] of the The Procalcitonin And Survival Study (PASS) Group*.

[1]Copenhagen HIV Programme at the University of Copenhagen; [2]Department of Clinical Microbiology at Copenhagen University Hospital Hvidovre; [3]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Glostrup; [4]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Hillerød; [5]Diagnostic Centre at Copenhagen University Hospital Rigshospitalet; [6]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Gentofte; [7]Department of Anesthesia and Intensive Care at Aarhus University Hospital in Skejby; [8]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Hvidovre; [9]Royal Free Hospital School of Medicine in London; [10]Department of

Anesthesia and Intensive Care at Copenhagen University Hospital Herlev; [11]Department of Anesthesia and Intensive Care at Copenhagen University Hospital in Roskilde; [12]Department of Anesthesia and Intensive Care at Aarhus University Hospital in Aarhus; [13]Department of Clinical Microbiology at Copenhagen University Hospital Herlev; [14]Department of Infectious Diseases at Copenhagen University Hospital Rigshospitalet. All except[9] are from Denmark. [9] is from England.

*Participating investigators are listed in the appendix.

Running Title: Broad-Spectrum Antibiotics and Renal Failure in Critically Ill Patients

Keywords: Antibiotics – Renal Failure – Sepsis – Intensive Care

Abstract

Objectives: To explore whether a strategy of more intensive antibiotic therapy leads to emergence or prolongation of renal failure in intensive care patients.

Design: Secondary analysis from a randomized antibiotic strategy trial (the PASS study). The randomized arms were conserved from the primary trial for the main analysis.

Setting: Nine mixed surgical/medical intensive care units across Denmark.

Participants: 1200 adult intensive care patients, 18+ years, expected to stay +24 hours. Exclusion criteria: Bilirubin >40 mg/dL. Triglycerides >1000 mg/dL, Increased risk from blood sampling, pregnant/breast feeding and psychiatric patients.

Interventions: Patients were randomized to: guideline-based therapy ('standard-exposure'-arm), or to guideline-based therapy supplemented with antibiotic escalation whenever procalcitonin increased on daily measurements ('high-exposure'-arm).

Main outcome measures: Primary endpoint: estimated GFR<60 ml/min/1.73 m2. Secondary endpoints: a) delta eGFR after starting/stopping a drug, b) RIFLE criterion Risk "R". Analysis was by intention to treat.

Results: 28-day mortality was 31.8% and comparable (Jensen et al, CCM 2011). A total of 3672/7634 (48.1%) study days during follow-up in the 'high-exposure' vs. 3016/6949 (43.4%) in the 'standard-exposure'-arm were spent with eGFR <60 ml/min/1.73m2, p<0.001. In a multiple effects model, piperacillin/tazobactam was identified as causing the lowest rate of renal recovery of all antibiotics: 1.0 ml/min/1.73 m2 per 24h while exposed to this drug [95% CI: 0.7 – 1.3 ml/min/1.73 m2/24h] vs. meropenem: 2.9 ml/min/1.73 m2/24h [2.5 – 3.3 ml/min/1.73 m2/24h]); after discontinuing piperacillin/tazobactam, the renal recovery rate increased: 2.7 ml/min/1.73 m2 /24h [2.3 – 3.1 ml/min/1.73 m2 /24h]). eGFR<60 ml/min/1.73m2 in the two groups at entry and at last day of follow-up was 57% vs. 55% and 41% vs. 39%, resp.

Conclusions: Piperacillin/tazobactam was identified as a cause of delayed renal recovery in critically ill patients. This nephrotoxicity was not observed when using other beta-lactam antibiotics.

Trial registration ClinicalTrials.gov identifier NCT00271752.

Introduction

Frequent complications to sepsis are organ failure, especially respiratory failure and renal failure [1-3]. Critically ill patients are more vulnerable to organ-related drug toxicities than less severely ill patients[4]. Randomized trials assessing safety of broad-spectrum antibiotics in intensive care settings are generally scarce, do not have sufficient statistical power for assessing organ failure endpoints, and do often not include defined kidney organ failure endpoints[5-7]. Data on renal failure endpoints are also sparse in the published trials from other patient populations, and since the absolute risk of renal failure is low for these patients, analyses may likely have been underpowered[8-12].

To our knowledge, randomized trials comparing 'high exposure' vs. 'standard exposure to antibiotics' and specifically addressing whether these interventions affect the occurrence and duration of kidney failure have not been done before in intensive care settings.

In this secondary analysis from a randomized trial, the PASS study[13], we aimed to explore whether a strategy of more intensive antibiotic therapy leads to adverse renal outcomes within 28 days after recruitment.

In our study population (and often in severely infected ICU patients), a bacterial hit has resulted in acute onset renal failure, and this bacterial hit (and related organ failure) is often the reason for ICU admittance. In such situations, with the correct treatment of the underlying infection, we expect renal function to recover. "Lack of recovery" is a non-desirable situation, which may be very serious for the patient. We wanted to explore this, and realizing, RIFLE/AKIN could not capture

this, we have used eGFR<60 ml/min/1.73 m^2 as the primary endpoint and examined this from different angles (eGFR<60 ml/min/1.73 m^2 at day 7, days with ml/min/1.73 m^2 . The multiple effects model was built to capture actual estimates of renal function improvement using different antibiotics and adjusting for other known or suspected causes of renal dysfunction.

Secondly, if renal failure was observed from the 'high exposure' approach, to identify one or several of the antibiotics used in this trial as the cause of such a renal failure.

Methods

Trial design and participants

PASS is a multicentre randomized controlled trial in Denmark 2006-9 in 1200 adult critically ill patients, expected to stay in one of the nine participating mixed medical/surgical intensive care units ≥24 hours; the CONSORT trial diagram is displayed in supplementary figure 1. Patients were randomized 1:1 either to treatment according to international guidelines: 'standard exposure arm', or to same guidelines but supplemented with daily drug-escalation initiated upon procalcitonin increases ('high exposure'-arm); 28-day mortality was 31.8% and comparable between the two groups, as reported[13].

To be eligible, patients had to be ≥18 years, enrolled within 24 hours of admission to the intensive care unit and have an expected intensive care-admission length of ≥ 24 hours. Patients with known bilirubin >40 mg/dL and triglycerides >1000 mg/dL (not suspensive) were not eligible (interference with procalcitonin measurements), as were patients who were judged to be at an increased risk from blood sampling. The inclusion criteria were broad since infection is frequent and often causes complications in the patient group and to increase the external validity of the results. The person or next of kin gave informed consent. The study protocol was approved by the regional ethics committees in Denmark (H-KF-272-753) and adheres to the Helsinki declaration, revised in Seoul 2008.

In the present analyses we explored presence and duration of renal failure as well as change in renal function during the observed time. Endpoints are defined in *statistical analysis* below. Patients were followed until day 28. The primary trial protocol and the analysis plan is available in the online supplement. Analysis was by intention to treat: NCT00271752.

Randomization and masking

Randomization was performed 1:1 using a computerized algorithm created by the database manager (JK) with concealed block-size, pre-stratified for site of recruitment, initial APACHE-II and age (entered in an encrypted screening form in a password protected website); investigators were masked to assignment before, but not after, randomization. All investigators were trained by the coordinating centre and had to register in an investigator-database. Investigators, treating physicians and the coordinator were unaware of outcomes during the study, as were they of all procalcitonin measurements in the 'standard exposure' (control)-group.

Antibiotic therapy in the two arms

The investigators enrolled participants and assigned the 'high exposure group' participants to the intervention. In the 'standard exposure' group, the antimicrobial treatment was guided according to current clinical guidelines[14], based on clinical assessment, microbiology and radiology among other parameters, as described elsewhere[13]

In the 'high exposure' group, the use of antimicrobial interventions was guided by the same clinical guidelines as in the 'standard exposure' group to ascertain the best standard of care therapy for all patients, and additionally antimicrobial interventions were initiated whenever procalcitonin levels were not decreasing at a pre-defined pace (supplementary figure 2) and diagram D1 in the online supplement where a site-adjusted local guideline is displayed.

Measurements, data collection and follow-up

Blood samples for biomarker measurement were made daily in the intensive care unit, beginning immediately after randomization. The assay used was the Kryptor®-PCT. Organ failure and antibiotic exposure was followed up for until 28 days or death, as described[13]. Mortality was followed via the National Patient Register in which all deaths in Denmark are registered within 14 days. Good Clinical Practice guidelines were applied. The regional ethics board approved the protocol (H-KF-01-272-753).

Statistical analysis

The primary endpoint was 'estimated GFR<60 ml/min/1.73 m^2' and several analyses were made to explore this: 'days with estimated GFR<60 ml/min/1.73 m^2', 'risk of estimated GFR<60 ml/min/1.73 m^2 on day 1-7'. Secondary endpoints were a) delta eGFR after starting/stopping a drug, b) RIFLE-criteria *Risk* 'R', *Injury* 'I' and *Failure* 'F' www.adqi.net.Other endpoints explored were 'ever' blood-urea level ≥20 mmol/L and eGFR<30.

The multiple effects eGFR 'slope' analyses, were adjusted for the following variables: treatment arm ('high exposure' vs. 'standard exposure'), age (≥65 vs. <65 years), gender, baseline APACHE II score (≥20 vs. <20), degree of host response/infection at baseline (severe sepsis/septic shock vs. milder or no infection as defined[15]), the eGFR at initiation of the investigated antibiotic, and finally, whether the patient at baseline was considered to be 'surgical' or 'medical'.

Comparisons were made between treatment arms using Students t-tests (for normal distributed continuous data) and Mann-Whitney U-tests (for non-normally distributed continuous data). Chi-squared tests and logistic regression models were used to test categorical variables. Time-to-event analyses comparing the 'high exposure' group with the 'standard exposure' group were performed using Kaplan-Meier plots and Cox proportional hazards models. Interactions were explored whenever an interaction could be rationally expected according to background literature, for the

multivariate models performed. Statistical analyses were performed using STATA Version 10.2, and SAS version 9.1. All reported p-values are 2-sided using a level of significance of 0.05.

Sample size

A multivariate approach power calculation was made: The summed squared correlations (Σrho^2) to the risk of the antibiotic drug investigated, was set to 0.3. The frequency of the endpoint in the 'standard exposure' group was set to 20%, the sample size was set to 1200, and the frequency of the exposure was set at 30%, which resulted in a detection limit for odds ratio of ≥1.5 (or ≤0.67).

Results

Baseline characteristics

Nine sites included 1200 persons between 09/01/06 and 02/06/09. Eighty-three percent of the patients were assessed by the investigator to have an infection at baseline and 81% of the patients suffered from chronic co-morbidity. Supplementary table 1 briefly summarizes baseline characteristics. Mortality was comparable between the two groups, as reported[13].

Follow-up

Follow-up for renal measures during the 28-day study period was made on 9,348 days in the 'standard-exposure' group of 10,755 days alive and admitted to hospital (86.9%) vs. 9,866 of 11,380 days in the 'high exposure group' (86.7%). If time after discharge from hospital (where no S-creatinine values were determined) until day 28 was included, the percentage of days with assessment of renal failure was 71.2% (9,348/13,130 days) vs. 73.8% (9,866/13,377 days)."

Use of Antibiotics

The antibiotics used most while admitted to the ICU were piperacillin/tazobactam, cefuroxim, meropenem and ciprofloxacin, and there was a substantial higher use of piperacillin/tazobactam and ciprofloxacin in the 'high exposure' arm (supplementary table 2). Vancomycin was used to a lesser extent in both groups and aminoglycosides and colistin were used rarely in both groups.

The median length of an antibiotic course was prolonged using the 'high exposure'-algorithm (6 days (IQR 3, 11) vs. 4 days (IQR 3, 10), p=0.004.

Renal failure in the originally randomized study arms

The % of days within day 1-28 with eGFR≤ 60 ml/min/m^2 was 48% in the 'high exposure' arm vs. 43% in the 'standard exposure' arm, p<0.0001. Results in table 1 are estimated eGFR values, based on actual measured S-creatinine values; results regarding days with eGFR were comparable if using the 'last observation carried forward' approach (not shown). RIFLE-criterion 'R' occurred more often within day 1-28 in the 'high exposure' arm than the 'standard exposure' arm: 209 patients vs. 170 patients, p=0.02, as did blood urea levels exceeding 20 mmol/L: 253 (43.4%) vs. 217 (37.4%), p=0.04.

The frequency of renal failure on the last day of follow-up was comparable between the arms (table 2), underlining that the results depicted in table 1 reflect a temporary extension of duration of renal failure in the "high exposure group" and furthermore that this observation is not explained by premature discharge of renally incompetent patients in the 'standard exposure' arm.

Glomerular Filtration Rate changes and exposure to certain antibiotics

Comparison of the eGFR of all patients (both study arms) for the first ten days after starting on the most frequently used betalactam antibiotics showed that the slowest recovery of renal function was

observed in patients on piperacillin/tazobactam as compared to patients on meropenem or cefuroxim (figure 1). A multiple effects model investigating the eGFR regression coefficient ('increase in eGFR') per day on these drugs confirmed that renal recovery was lowest in patients on piperacillin/tazobactam (table 3). Of note, renal recovery seems to be low in patients exposed to cefuroxim, but as displayed in fig. 1, this drug is given to patients with a relatively normal renal function (leaving few possibilities for 'recovery').

For the first five days following discontinuation of these drugs, adjusting for the same variables, eGFR increased at the highest rate in patients receiving piperacillin/tazobactam (table 3).

The frequency of eGFR<60 ml/min/1.73 m^2 on day 7 (or at death or last follow-up day) in the trial was 523/1200 = 43.6%. This endpoint was investigated in a forward censored ($p<0.1$) logistic regression. .. Use of piperacillin/tazobactam and other frequently used beta-lactam drugs for at least three days within these first seven days, as well as known and suspected predictors of renal failure were explored in a multivariable logistic regression analysis. Five independent predictors of renal failure on day 7 were identified: Age above 65 years, APACHE II score >20, Charlson´s co-morbidity score ≥2, estimated GFR at baseline and use of piperacillin/tazobactam for at least 3 days within the first 7 days (table 4) Excluding all patients who died within the first seven days, excluding all patients with invasive fungal infection on day 1-28, combining the betalactam exposure with exposure to flour-quinolone exposure (data not shown) or 4) adding 'Alert-procalcitonin' at baseline as a variable, did not alter the signal (data not shown).

Discussion

Principal findings

We observed that the duration of renal failure is prolonged in critically ill patients randomized to receive high exposure to broad-spectrum antibiotics and escalated diagnostic work-up according to a biomarker-strategy, compared to patients randomized to receive standard care according to

guidelines regarding use of antibiotics and diagnostics. This difference in renal function was mainly confined to a prolongation of existing renal dysfunction, since there was only a moderate, although significant, difference in de novo acute renal failure.

To our knowledge, this study provides the first clinical report to inform this critical issue within ICU medicine. Firstly, the study was a randomized, good clinical practice controlled trial with a high sample size for comparison of organ failure, and the patients' baseline characteristics in general and specifically regarding renal parameters, were comparable. Secondly, the rate of follow-up, although not complete for the entire period, was high and equal among the groups and the rate of renal failure on the last day of follow-up in the two groups was comparable. Thus, the observed increased risk of persistent renal failure in the "high-exposure group" is attributable to this intervention in some way.

The intervention consisted of an increased number of culture samples, a proposed initiative to do further diagnostic imaging (no observed difference) and a rapid and aggressive antibiotic escalation with certain drugs, which was documented to be of substantial extent (supplementary table 2). As a moderate increase in microbiologic sampling would not cause renal failure, and since there was no observed increase in diagnostic imaging, these interventions seems implausible reasons to explain the observations depicted in table 1.

This leaves us with the documented escalation in use of piperacillin/tazobactam and ciprofloxacin as possible explanations. Before concluding, that the observed renal dysfunction was caused directly by one (or both) of these drugs, we wanted to exclude the possibility that the results had appeared because of a derived effect of an increase in fungal infections. Fungal infections have been linked to broad-spectrum antibiotics[16], and renal failure is a well-known complication to some antifungals[17]. However, excluding all patients with invasive fungal infections did not alter the results.

Based on these results, and after having excluded other potential explanations, we realized

that nephrotoxicity from piperacillin/tazobactam and/or ciprofloxacin was the most plausible explanation of the observed renal dysfunction. To further substantiate this, several analyses were conducted. A multiple effects model was built to examine the GFR in the days after administration of different frequently used drugs. This model included the five most often administered antibiotics, including piperacillin/tazobactam, meropenem, cefuroxim, ciprofloxacin and vancomycin along with other known and suspected causes of renal failure. In this model, the use of piperacillin/tazobactam was associated with a striking low rate of GFR-improvement, compared to the other drugs investigated. Intriguingly, this adverse effect appears to be reversible, since patients in whom, piperacillin/tazobactam was discontinued, had the fastest improvement in renal function as compared with patients on other antibiotic courses. Several sensitivity analyses were performed with findings consistent with this observation.

Comparison with other studies

Although clinical evidence regarding renal failure according to use of piperacillin/tazobactam in ICU patients has been limited, the influence of piperacillin on renal function has been investigated in healthy volunteers in laboratory experiments. In a cross-over experiment, the influence on drug clearance from concurrent administration of piperacillin and flucloxacillin was estimated[18]. The authors observed that flucloxacillin clearance was reduced to 45% [90% CI: 40 – 50%] when piperacillin was administered simultaneously, whereas piperacillin clearance was unaffected by concurrent flucloxacillin administration. Time-clearance slope modeling identified competitive inhibition of renal tubular secretion as the most likely explanation. Piperacillin-induced reduction of imipenem clearance[19] and of tazobactam clearance has also been found[20], and a high correlation between creatinin clearance and piperacillin clearance has been documented[21], and thus, it is plausible that piperacillin specifically causes nephrotoxicity.

Additionally, the published randomized trials comparing piperacillin/tazobactam with other beta-lactam drugs in intensive care unit settings are scarce, underpowered for assessment of renal failure endpoints and do generally not address renal endpoints[5-7]. Trials from other settings: haematological patients, diabetes patients, and surgical settings do generally not investigate renal failure endpoints, and in the few (non-ICU) trials that do report kidney endpoints, the total frequency of these makes the power to avoid type II error very low (diagram D2, online supplement).

Strengths and weaknesses of the study

Although our study is performed on analyses from a large randomized good clinical practice controlled trial with a stringent methodology and a high level of follow-up, there are limitations that deserve mentioning: First, follow-up for organ-related measures was not complete, although we followed patients for all blood samples done in 1) the hospital, at which they were initially recruited, 2) other hospitals in Denmark, where we had electronic access to blood samples. However, patients who continued to suffer from renal failure when discharged from hospital, were out of reach for follow-up for their renal function. Of note, the fraction of patients with remaining renal failure at time of discharge was comparable between the two groups (table 2), and hence it is unlikely that this lack of ability to ascertain renal outcome contributed to our main findings.

Second, eGFR may not be an accurate measure of creatinine clearance, as recently documented by Martin et al. [22]. However, even though this measure is not accurate to describe the creatinine clearance, changes in eGFR reflect changes in renal function, as validated, and is closely correlated to outcome[23]. Additionally, we found that eGFR<60 ml/min/1.73 m^2 on day 7 is a strong independent predictor of mortality.

Third, the study was a post hoc analysis using a previously published trial as material. We have tried to compensate for this by writing a detailed analysis-plan based on the hypotheses, we wanted

to test, before analysis. Third, although the sample size was relatively large compared to most other randomized trials in this setting, the sample size for these secondary analyses were based on the assumption of 25% renal failure in the 'standard exposure group' and a relative risk of 1.25 in the 'high exposure group'. The observed numbers were 21% and 1.22 which calls for a slightly higher sample size. However, the sample size needed to show the differences observed in the multivariable analyses was far smaller, and since these analyses confirmed the main findings, we do not think the results are due to chance.

In this trial, for the first time ever to our knowledge, random allocation to high exposure to broad-spectrum antibiotics in the intensive care unit has been systematically applied according to a systematic algorithm and this resulted in prolongation of renal failure. The results were confirmed when excluding patients with fungal infections, and a multiple effects model revealed a particularly low renal recovery in patients while piperacillin/tazobactam was administered and a remarkable recovery when discontinuing this drug; a finding that was specific for this drug. Several other crude and adjusted models likewise confirmed the findings. Finally, the results from this trial are supported by human experimental studies.

Conclusion

In conclusion, the use of piperacillin/tazobactam caused a delayed renal recovery in critically ill patients, and renal function improved after discontinuation of the drug. However, the study is not designed to investigate d*e novo* emergence of renal failure, since the lowest renal function is at baseline in most patients. We cannot within the sample size and follow-up time of this trial establish whether the use of piperacillin/tazobactam, in some cases causes persistent renal failure, and thus, further research to explore this is warranted. We think this impact on renal function is more likely caused by a toxic effect on the renal tubule than by a lack of effect towards the infection, since this

drug is independently associated with a high chance of survival in other infected populations[8], and we must emphasize that our findings are strictly confined to critically ill patients.

Contributors

JUJ designed the study, made the data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, and drafted and the paper. He is guarantor. JUJ, ZF and JK cleaned and analysed the data. JL, BL, LH, MHB, TM, MHA, KJT, JL, MS, HT, PS-J, AØL, DGS, NR, KT, PCF, KML, NED, MEJ, LR, CØ, ZF, JK and JG made input study design, data collection tools and analysis plan and on the manuscript. JUJ implemented the trial at the centers. All members of the Procalcitonin And Survival Study (PASS) Group assisted in designing the trial. The members of the PASS study group are as follows: Central Coordinating Centre - J.U. Jensen, B. Lundgren, J. Grarup, M.L. Jakobsen, S. S. Reilev, M. Kofoed-Djursner, J. D. Lundgren; Regional Coordinating Centres - Hvidovre - J. Løken, M. Steensen; Gentofte - T. Mohr, K. Thornberg, K. Thormar; Hillerød - L.Hein, M. Bestle; Glostrup - D. Strange, A.Ø. Lauritsen; Herlev - H. Tousi, P. Søe-Jensen; Roskilde - N. Reiter, N.E. Drenck; Skejby - M.H. Andersen, P. Fjeldborg; Århus - K.M. Larsen; Data Management & Statistical Centre - Z. Fox, J. Kjær, D. Kristensen; Procalcitonin Analysis & Logistics Centre - J.U.Jensen, B. Lundgren, M. B. Rasmussen, C. S.v.Hallas, M. Zacho, J. Iversen, T. Leerbeck, M. Jeppesen, K.S. Hansen, K.B. Jensen; Data and Safety Monitoring Board - H. Masur (Chair), J. Chastre, H. Schønheyder, C. Pedersen; Clinical Microbiology Management – B. Lundgren, J. D. Knudsen, A. Friis-Møller, K. Schønning, A. Lester, H. Westh, G. Lisby, J.K. Møller, B. Bruun, J.J. Christensen, C. Østergaard, M. Arpi, K. Astvad, M.D. Bartels, J. Engberg, H. Fjeldsøe-Nielsen, U.S. Jensen; PASS Site Clinical Investigators (numbers of recruited persons are in parentheses) - Glostrup (290) – L. Hein, T. Mohr, D. G. Strange, P. L. Petersen, A. Ø. Lauritsen, S. Hougaard, T. Mantoni, L. Nebrich, A. Bendtsen, L.H. Andersen, F. Bærentzen, Andreas Eversbusch, B. Bømler, R. Martusevicius, T.

Nielsen. P.M. Bådstøløkken, C. Maschmann, U. Grevstad, P. Hallas, A. Lindhardt, T. Galle, K. Graeser, E. Hohwu-Christensen, P. Gregersen, H.C. Boesen, L.M. Pedersen, K. Thiesen, L.C. Hallengreen, I. Rye, J. Cordtz, K.R. Madsen, P.R.C. Kirkegaard, L. Findsen, L.H. Nielsen, D.H. Pedersen, J.H. Andersen, C. Albrechtsen, A. Jacobsen, T. Jansen, A.G. Jensen, H.H. Jørgensen, M. Vazin; Gentofte (209) – L. Lipsius, K. Thornberg, J. Nielsen, K. Thormar, M. Skielboe, B. Thage, C. Thoft, M. Uldbjerg, E. Anderlo, M. Engsig, F. Hani, R.B. Jacobsen. L. Mulla, U. Skram; Herlev (154) – H. Tousi, P. Søe-Jensen, T. Waldau, T. Faber, B. Andersen, I. Gillesberg, A. Christensen, C. Hartmann, R. Albret, D.S. Dinesen, K. Gani, M. Ibsen; Hvidovre (148) – J. Løken, M. Steensen, J.A. Petersen, P. Carl, E. Gade, D. Solevad, C. Heiring, M. Jørgensen, K. Ekelund, A. Afshari, N. Hammer, M. Bitsch, J.S. Hansen, C. Wamberg, T.D. Clausen, R. Winkel, J. Huusom, D.L. Buck, U. Grevstad, E. Aasvang, K. Lenz, P. Mellado, H. Karacan, J. Hidestål, J. Høgagard, J. Højbjerg, J. Højlund, M. Johansen, S. Strande; Hillerød (138) – M. Bestle, S. Hestad, M. Østergaard, N. Wesche, S.A. Nielsen, H. Christensen, H. Blom, C.H. Jensen K. Nielsen, N.G. Holler, K.A. Jeppesen; Århus-Skejby (94) – M.H. Andersen, P. Fjeldborg, A. Vestergaard, O. Viborg, C.D. Rossau; Roskilde (90) – N. Reiter, M. Glæemose, M.B.Wranér, C.B. Thomsen, B. Rasmussen, C. Lund-Rasmussen, B. Bech, K. Bjerregaard, L. Spliid, L.L.W. Nielsen, N.E. Drenck; Århus-Centre (63) – K.M. Larsen, M. Goldinger, D. Illum, C. Jessen, A. Christiansen, A. Berg, T. Elkmann, J.A.K. Pedersen, M. Simonsen; Bispebjerg (14) H. Joensen, H. Alstrøm, C. Svane, A. Engquist.

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Competing interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that the trial was funded mainly by the Danish State (Danish Research Council) and : all authors state that they have no relationships with companies that might have an interest in the submitted work in the previous 3 years; their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and all authors have no non-financial interests that may be relevant to the submitted work.

Ethical approval

The study was approved by the ethics committee for Copenhagen and Frederiksberg community (now Ethics Committee for the Capitol Region): H-KF-01-272-753. Patient consent: We received written consent from the patient or the next of kin for trial inclusion.

Data sharing

No additional data available.

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Table 1: Prevalence and duration of kidney organ failure ('Standard exposure' group vs. 'High exposure' group)

	'Standard exposure' group (N=596)	'High exposure' group (N=604)	p-value
EstimatedGFR*: N. days (% of days from day 1 to 28 with values):			
Moderately-severely impaired: (eGFR: ≤60 mL/min/1.73 m^2)	3016 (43.4%)	3672 (48.1%)	<0.0001
Severely impaired: (eGFR ≤30 mL/min/1.73 m^2)	1445 (20.8%)	1910 (25.0%)	<0.0001
Severely impaired: (eGFR ≤30 mL/min/1.73 m^2), days from day 1 to 14	984 (20.0%)	1253 (23.5%)	<0.0001
'RIFLE' criteria, N patients (%) within day 1 to 28			
'R' reached	170 (28.5%)	209 (34.6%)	0.02
'I' reached	75 (12.6%)	92 (15.2%)	0.19
'F' reached	121 (20.3%)	150 (24.8%)	0.06
'R' or death	298 (50.0%)	327 (54.1%)	0.15
'I' or death	234 (39.3%)	252 (41.7%)	0.39
'F' or death	270 (45.3%)	287 (47.5%)	0.44
Urea			
Patients with a urea level ever ≥ 20 mmol/L (day 1-28); N (%)	217 (37.4%)	253 (43.4%)	0.04

*eGFR was assessed using the Cockcroft and Gault method [Ref: Cockcroft DW, Gault MH.: Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41]. Actual measured creatinin values were used. If using the 'last observation carried forward' approach regarding creatinin measurement to take into account that patients who died in renal failure should be counted as such, did not change the signal or the statistics of these analyses. 'R':Risk, 'I': Injury, 'F': Failure. Presence of renal failure according to 'RIFLE' was assessed using the guidelines developed by the acute dialysis quality initiative (www.adqi.net)

Table 2: Prevalence of kidney organ failure on the last day of follow-up ('Standard exposure' group vs. 'High exposure' group)

	'Standard exposure' group	'High exposure' group	p-value
Survivors and patients who had last creatinine measured>24 h before death:	**(N=432)**	**(N=438)**	
Renal failure (eGFR: ≤60 mL/min/1.73 m^2)	119 (27.6%)	137 (31.3%)	0.23
Patients who died (with last creatinine measured within 24 h before death):	**(N=150)**	**(N=145)**	
Renal failure (eGFR: ≤60 mL/min/1.73 m^2)	105 (70.0%)	99 (68.3%)	0.83
All patients with creatinine measurements	**(N=582)**	**(N=583)**	
Renal failure (eGFR: ≤60 mL/min/1.73 m^2)	224 (38.5)	236 (40.5)	0.51

*eGFR was assessed using the Cockcroft and Gault method [Ref: Cockcroft DW, Gault MH.: Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41]. Actual measured creatinin values were used.

Table 3. Multiple effects models investigating estimated GFR changes after starting and stopping beta-lactam antibiotics

		Unadjusted analysis		Multivariable analysis	
	Variable	**Regression coefficient (95% CI)**	**P-value**	**Regression coefficient (95% CI)**	**P-value**
After starting the drug					
Piperacillin/tazobactam	Per day more on piperacillin/tazobactam	1.39 (1.17, 1.60)	<0.0001	0.99 (0.71, 1.27)	<0.0001
Meropenem	Per day more on meropenem	2.74 (2.39, 3.09)	<0.0001	2.86 (2.45, 3.28)	<0.0001
Cefuroxim	Per day more on cefuroxim	1.91 (1.67, 2.16)	<0.0001	1.27 (0.90, 1.64)	<0.0001
After stopping the drug					
Piperacillin/tazobactam	Per day after stopping piperacillin/tazobactam	2.79 (2.35, 3.24)	<0.0001	2.70 (2.26, 3.14)	<0.0001
Meropenem	Per day after stopping meropenem	0.20 (-0.51, 0.91)	0.59	0.17 (-0.52, 0.86)	0.63
Cefuroxim	Per day after stopping cefuroxim	0.13 (-0.25, 0.50)	0.51	0.01 (-0.35, 0.37)	0.96

All multivariable analyses were adjusted for: treatment arm ('low exposure' vs. 'high exposure'), gender, age (≥65 vs. <65 years), APACHE II score (≥20 vs. <20), Clinically judged infection (severe sepsis/septic shock vs. milder or no infection), patient category (surgical vs. medical) and eGFR level at administration of the antibiotic, (1: <30 ml/min/1,73 m^2, 2: 31-60 ml/min/1,73 m^2, 3: >60 ml/min/1,73 m^2).

Table 4. Multivariable logistic regression: beta-lactam antibiotics and other risk variables vs. binary endpoint eGFR<60 ml/min/1.73m^2 on day 7.

	Unadjusted analysis		Multivariable analysis	
Variable	**Odds ratio (95% CI)**	**P-value**	**Odds ratio (95% CI)**	**P-value**
Other variables				
Age (≥65 vs. <65 years)	2.36 (1.86, 3.00)	<0.0001	1.85 (1.31, 2.60)	<0.0001
APACHE II score (≥20 vs. <20)	2.49 (1.90, 3.25)	<0.0001	1.64 (1.12, 2.41)	0.01
Severe sepsis/septic shock vs. milder or no infection	2.02 (1.59, 2,56)	<0.0001	1.16 (0.82, 1.66)	0.40
Auto-immune disease (Y vs. N)	1.31 (0.73, 2.33)	0.36	NI	-
Cancer (Y vs. N)	1.26 (0.88, 1.79)	0.21	NI	-
Charlson score (≥2 vs. <2)	1.72 (1.35, 2.18)	<0.0001	1.70 (1.21, 2.40)	0.002
Surgical (Y vs. N)	1.16 (0.90, 1.50)	0.24	NI	-
Body Mass Index (≥25 vs. <25)	1.57 (1.17, 2.12)	0.003	1.19 (0.78, 1.82)	0.41
Gender (Male vs. Female)	1.25 (0.99, 1.57)	0.06	1.28 (0.92, 1.78)	0.14
eGFR level at baseline				
>60 ml/min/1,73 m^2	Ref	-	Ref	-
31-60 ml/min/1,73 m^2	14.6 (10.2, 21.0)	<0.0001	11.7 (8.0, 17.0)	<0.0001
<30 ml/min/1,73 m^2	81.1 (51.2, 128.5)	<0.0001	65.9 (40.7, 106.6)	<0.0001
Beta-lactam antibiotics				
Piperacillin/tazobactam (≥3 vs. <3 days)*	2.32 (1.82, 2.96)	<0.0001	1.70 (1.18, 2.43)	0.004
Meropenem (≥3 vs. <3 days)*	0.99 (0.71, 1.37)	0.94	NI	-
Cefuroxim (≥3 vs. <3 days)*	0.73 (0.57, 0.94)	0.01	1.24 (0.85, 1.80)	0.26

All variables entered in the multivariable analysis were adjusted for the other variables in this model. *All beta-lactam drug exposures are (≥3 vs. <3 days within the first 7 days in the study). All variables with a p-value <0.2 were included in the multivariable model. NI: Not Included.

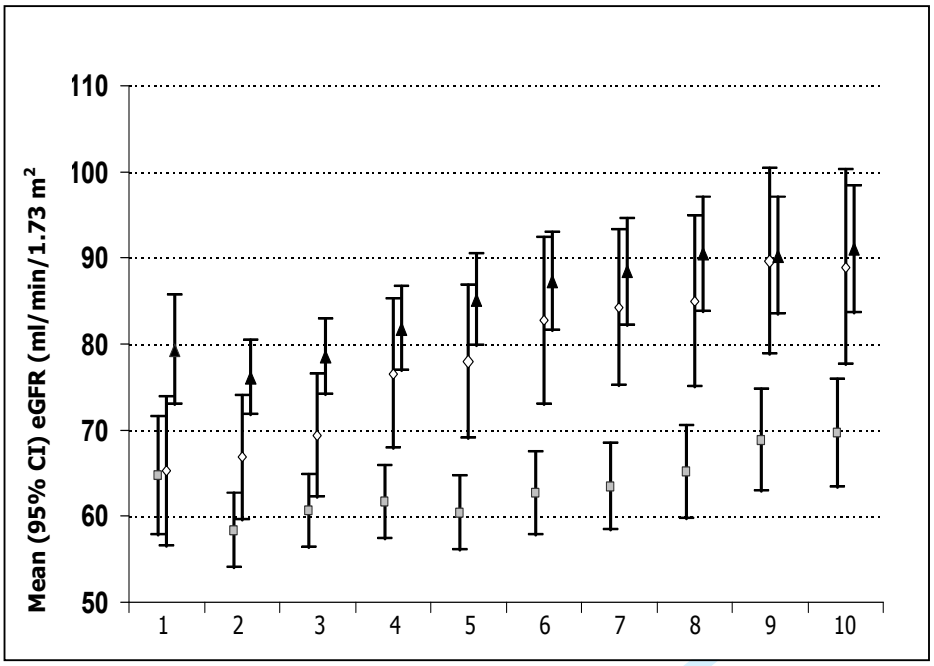
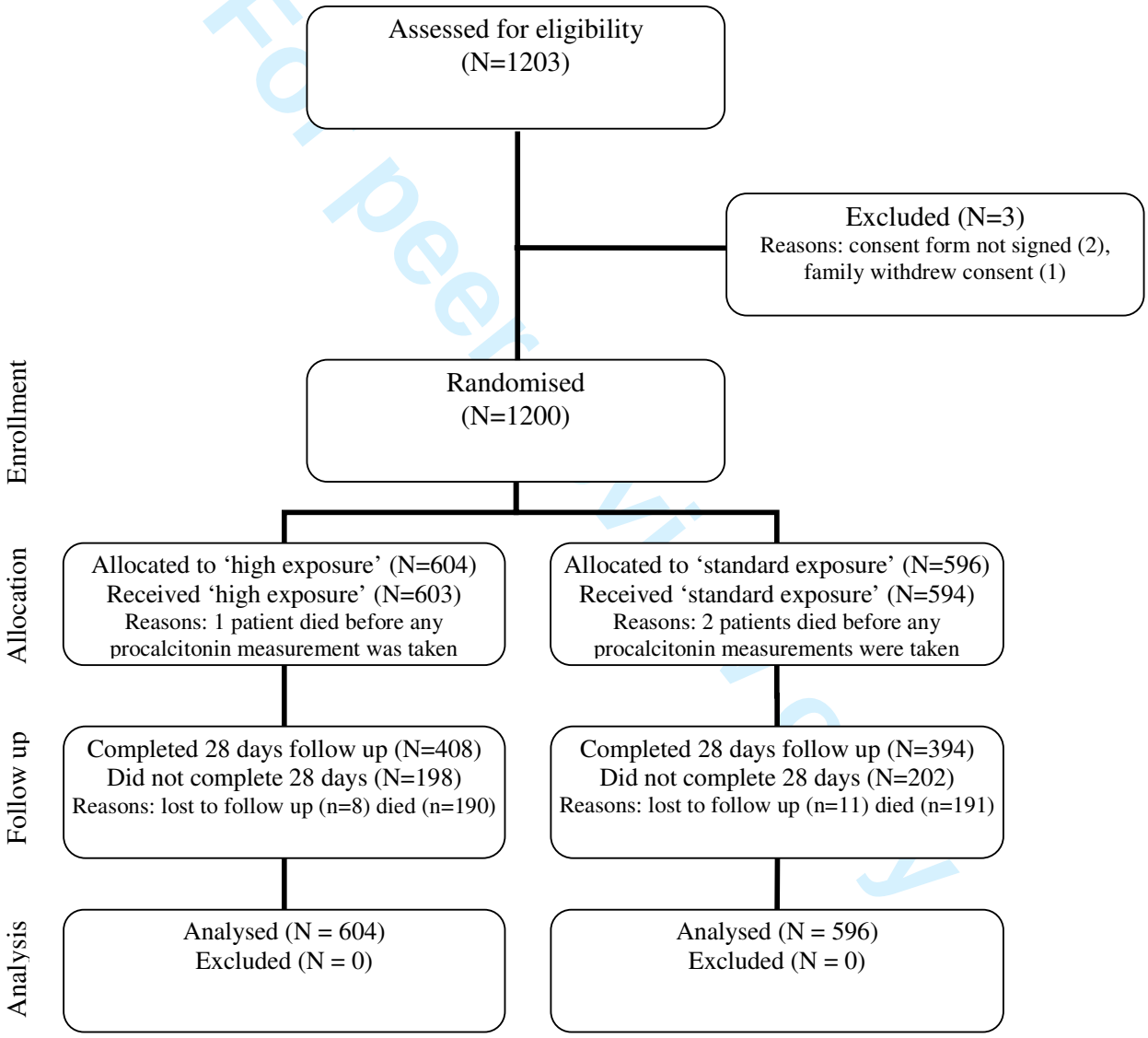
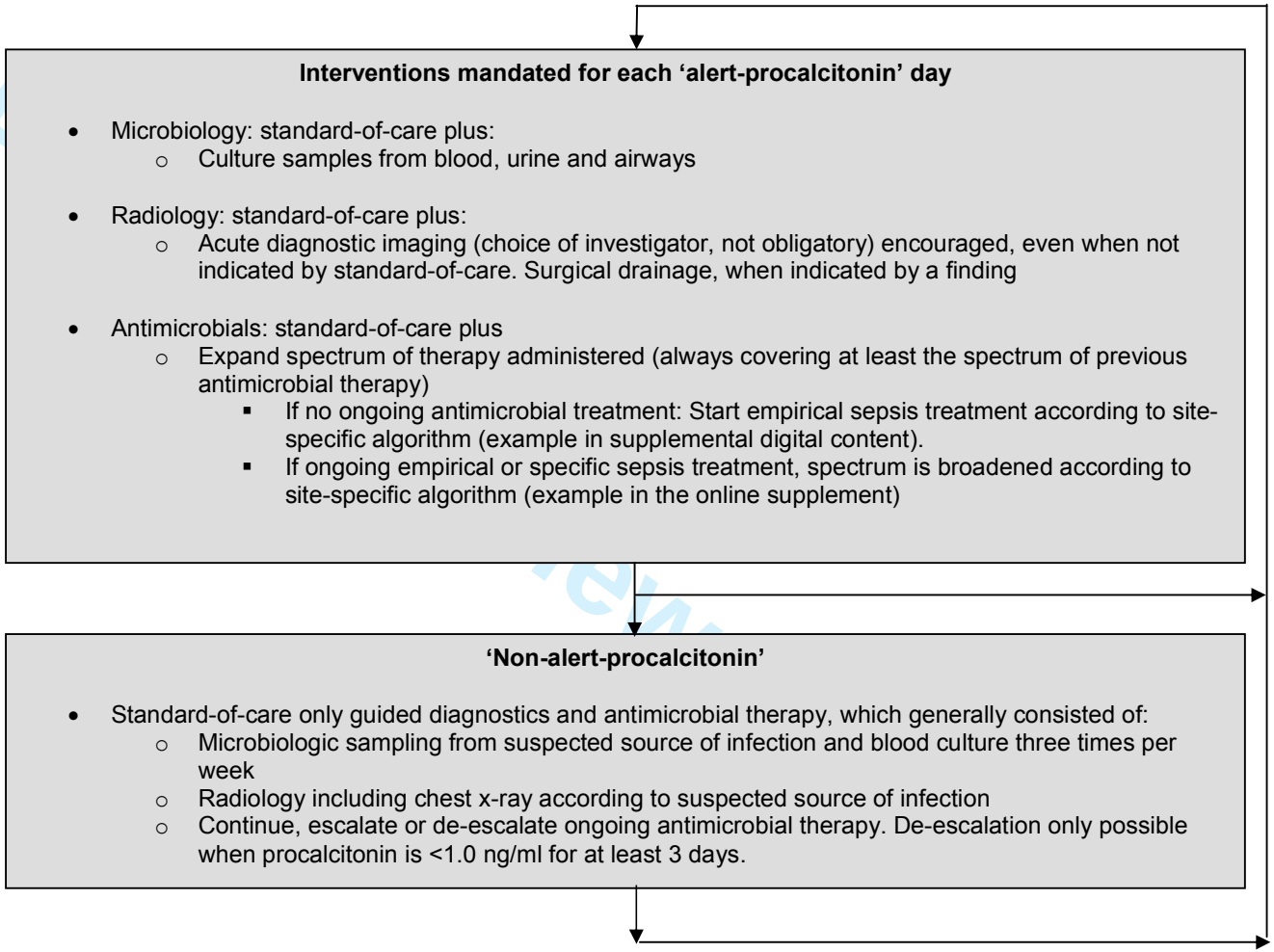


Figure 1. eGFR during ten days on cefuroxim, piperacillin/tazobactam and meropenem. ▲=cefuroxim; ▩ =piperacillin/tazobactam; ◇=meropenem.

Differences between eGFR in patients receiving piperacillin/tazobactam vs. meropenem: day 1 (p=0.78), day 2 (p=0.18), day 3 (p=0.09), day 4 (p=0.008), day 5 (p=0.001), day 6 (p=0.001), day 7 (p=0.0004), day 8 (p=0.005), day 9 (p=0.006), day 10 (p=0.02).



Supplementary Figure 1. Patient Flow Diagram of the trial


Interventions mandated for each 'alert-procalcitonin' day
Microbiology: standard-of-care plus:
Culture samples from blood, urine and airways
Radiology: standard-of-care plus:
Acute diagnostic imaging (choice of investigator, not obligatory) encouraged, even when not indicated by standard-of-care. Surgical drainage, when indicated by a finding
Antimicrobials: standard-of-care plus
Expand spectrum of therapy administered (always covering at least the spectrum of previous antimicrobial therapy)
If no ongoing antimicrobial treatment: Start empirical sepsis treatment according to site-specific algorithm (example in supplemental digital content).
If ongoing empirical or specific sepsis treatment, spectrum is broadened according to site-specific algorithm (example in the online supplement)
'Non-alert-procalcitonin'
Standard-of-care only guided diagnostics and antimicrobial therapy, which generally consisted of:
Microbiologic sampling from suspected source of infection and blood culture three times per week
Radiology including chest x-ray according to suspected source of infection
Continue, escalate or de-escalate ongoing antimicrobial therapy. De-escalation only possible when procalcitonin is <1.0 ng/ml for at least 3 days.

Supplementary Figure 1. General principles of procalcitonin-guided intervention.

At 'alert-procalcitonin' situation (≥ 1.0 ng/ml and not decreasing by at least 10% from the previous day), interventions were obligatorily conducted according to an algorithm with specific instructions for intervention, which was adapted to the antimicrobial guidelines on the site. Antimicrobials were daily adjusted according to 1) present and previous procalcitonin values, 2) infectious state of the patient (clinical presentation, microbiology, radiology etc.) and 3) history of antimicrobial use. Procalcitonin-guided antimicrobial escalation was mandatory, except when 1) there was a clear contra-indication for administering it or 2) microbiology "explaining the infectious presentation of the patient" was announced (same date) leading to specific therapy. Standard-of-Care antimicrobial diagnostics and treatment was not waived in the 'high exposure arm (nor the 'standard exposure'arm) to assure patient safety. According to the standard-of-care principle, all patients with septic shock were treated at the onset of hypotension with antimicrobials covering >95% of the causes of this condition in our hospitals. Awaiting procalcitonin results/low procalcitonin levels was not considered a plausible reason to withhold antimicrobial treatment. The treating physician was reminded daily via phone from the coordinating centre at each 'alert-procalcitonin' to intervene. In the 'standard exposure' arm, procalcitonin measurements were not available.

Supplementary Table 1. Baseline characteristics of the study participants.

	'Standard exposure' group (n=596)	'High exposure' group (n=604)	Overall (n=1200)
Age, years - median (IQR)	67 (58–75)	67 (58–76)	67 (58–76)
Male sex – no. (%)	333 (55·9%)	330 (54.6%)	663 (55.3%)
Body Mass Index, kg/m2 – median (IQR)	24.7 (22.0–27.8)	25.0 (22.5–28.7)	24.8 (22.2–27.9)
APACHE II Score - median (IQR)	18 (13–24)	18 (13–25)	18 (13–24)
Chronic co-morbidity* - no. (%)			
No chronic co-morbidities	102 (17.1)	123 (20.4)	225 (18.8)
Kidney function and electrolytes			
Creatinin, µmol/L - median (IQR)	119 (78, 197)	119 (75, 208)	119 (76, 202)
eGFR, mL/min/1.73m^2 – median (IQR)	51.4 (29.2, 80.5)	49.4 (25.4, 82.6)	50.2 (27.1, 81.5)
Carbamid, mmol/L - median (IQR)	10.3 (6.5, 17.0)	10.6 (6.3, 18.1)	10.5 (6.4, 17.4)
Na^+, mmol/l - median (IQR)	138 (134, 141)	137 (134, 141)	138 (134, 141)
K^+, mmol/l - median (IQR)	4.0 (3.7, 4.4)	4.0 (3.6, 4.5)	4.0 (3.6, 4.4)
pH - median (IQR)	7.29 (7.21–7.39)	7.29 (7.20–7.38)	7.29 (7.20–7.38)
Dialysis required, patients (%)	88 (14.8%)	86 (14.2%)	174 (14.5%)
Indicators of severity (non-renal)			
Temperature, ^{0}C - median (IQR)	37.2 (36.4–38.0)	37.3 (36.5–38.1)	37.3 (36.4–38.0)
Mean arterial pressure, mmHg - median (IQR)	71 (60–84)	72 (63–85)	71 (62–84)
Heart frequency - median (IQR)	100 (82–116)	100 (84–117)	100 (83–117)
Need for vasopressor/inotropic drug† - n (%)	315 (52.9)	326 (53.4)	641 (53.4)
Mechanical ventilation used - n (%)	401 (67.3%)	401 (66.4%)	802 (66.8%)
Biomarkers			
Alert-PCT § – no. (%)	279 (47.0)	312 (51.7)	591 (49.4)
Leukocytes, x10^9 – median (IQR)	13.0 (8.8–18.1)	12.4 (8.0–18.1)	12.8 (8.4–18.1)
C-reactive protein, mg/L – median (IQR)	131 (40–234)	137 (40–253)	135 (40–241)

Interquartile range (IQR). Acute Physiology and Chronic Health Evaluation II score (APACHE II) ranges from 0 to 71. *Chronic co-morbidity: Earlier diagnosed via hospital admission: heart failure, lung disease, cancer, diabetes, alcohol abuse, chronic infection, neurological disease, renal diseases, liver disease, gastro-intestinal disease, autoimmune disease, cancer and psychiatric disorders. †Vasopressors/inotropic drugs are considered to be epinephrine, nor-epinephrine, dopamine and dobutamine. ‡ Infections were rated according to the ACCP/SCCM definitions; investigators were trained in using them. §Alert-PCT: Procalcitonin-level not decreasing by at least 10% from the previous day and above 1.0 ng/ml. If only one measurement is available: Absolute procalcitonin-level above 1.0 ng/ml. A comprehensive baseline table is available in the primary publication from this material[13].

Supplementary Table 2. Consumption of antimicrobials during follow-up

	Standard exposure (n=596)	High exposure (n=604)	p-value
Consumption of antimicrobials			
Pip/tazo used within 28 days (DDD)	1893	2925	-
Proportion of days[a] followed where Pip/tazo was used	0.00 (0.00 – 0.33)	0.11 (0.00 – 0.56)	<0.001
Meropenem used within 28 days (DDD)	2174	2480	-
Proportion of days[a] followed where meropenem was used	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.07)	0.23
Cefuroxim used within 28 days (DDD)	4369	3390	-
Proportion of days[a] followed where cefuroxim was used	0.11 (0.00 – 0.39)	0.04 (0.00 – 0.29)	<0.001
Ciprofloxacin used within 28 days (DDD)	6210	8382	-
Proportion of days[a] followed where ciprofloxacin was used	0.21 (0.00 – 0.71)	0.33 (0.04 – 0.88)	<0.001
Number (%) ICU days spent with at least three antimicrobials	2721 (57.7%)	3570 (65.5%)	0.002

ICU: Intensive care unit. [a]This comparison was made with complete follow-up for 28 days (if patients were discharged from ICU, they were followed for antimicrobial use in all hospital admissions in Denmark). Pip/tazo: piperacillin/tazobactam. DDD: Defined Daily Dose administered within day 1-28. Parts of this table is also available in the primary publication on this material[13]. It is included in the present report since it is crucial for interpretation of the results.

Supplementary table 3: Cox proportional hazards models investigating predictors of mortality after ten days

	Unadjusted analysis		Multivariable analysis	
Variable	**Hazard ratio (95% CI)**	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Treatment arm ('High exposure vs. 'Standard exposure)	0.97 (0.72, 1.31)	0.86	0.93 (0.69, 1.26)	0.63
Hospital:				
1	Ref	0.11	Ref	0.37
2	0.63 (0.19, 2.05)		0.50 (0.15, 1.66)	
3	0.54 (0.17, 1.75)		0.49 (0.15, 1.63)	
4	0.86 (0.26, 2.81)		0.65 (0.19, 2.21)	
5	0.56 (0.16, 1.88)		0.45 (0.13, 1.56)	
6	0.71 (0.21, 2.37)		0.63 (0.18, 2.12)	
7	0.79 (0.23, 2.72)		0.66 (0.18, 2.40)	
8	0.43 (0.11, 1.53)		0.34 (0.09, 1.26)	
9	0.23 (0.05, 1.02)		0.27 (0.06, 1.26)	
Gender (Female vs. Male)	0.80 (0.59, 1.08)	0.14	0.77 (0.57, 1.05)	0.10
Age (≥65 years vs. <65 years)	1.96 (1.42, 2.69)	<0.0001	1.86 (1.34, 2.58)	<0.0001
APACHE II score (≥20 vs. <20)	1.77 (1.31, 2.39)	<0.0001	1.35 (0.98, 1.87)	0.07
Infection at baseline (Severe Sepsis or septic shock vs Milder or no infection)	1.31 (0.97, 1.76)	0.08	1.17 (0.84, 1.64)	0.35
Surgical patient (Yes vs. No)	0.78 (0.57, 1.06)	0.11	0.76 (0.55, 1.05)	0.09
Date recruited (01/01/08 to 02/06/09 vs. 09/01/06 to 31/12/07)	1.11 (0.81, 1.53)	0.50	1.18 (0.84, 1.67)	0.34
eGFR ever <30 mL/min/1.73 m^2 over the first ten days (Yes vs. No)	1.81 (1.34, 2.45)	<0.0001	1.47 (1.06, 2.04)	0.02

Table T1: Cox proportional hazards models investigating predictors of 28 day 'all cause' mortality

	Unadjusted analysis		Multivariable analysis	
Variable	**Hazard ratio (95% CI)**	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Gender (Male vs. Female)	1.11 (0.90, 1.35)	0.34	NI	-
Age (≥65 years vs. <65 years)	2.04 (1.64, 2.54)	<0.0001	1.84 (1.47, 2.30)	<0.0001
APACHE II score (≥25 vs. <25)	1.89 (1.53, 2.33)	<0.0001	1.46 (1.17, 1.82)	0.001
Severe Sepsis/septic shock vs. Milder or no infection)	1.41 (1.15, 1.72)	0.001	1.28 (1.04, 1.58)	0.02
Surgical patient (Yes vs. No)	0.66 (0.52, 0.85)	0.001	0.64 (0.50, 0.82)	0.001
Cancer (Yes vs. No)	1.14 (0.85, 1.55)	0.38	NI	-
Charlson score (≥2 vs. <2)	1.46 (1.19, 1.80)	<0.0001	1.43 (1.14, 1.81)	0.002
eGFR <60 mL/min/1.73 m^2 on day 7 (Yes vs. No)	2.14 (1.74, 2.63)	<0.0001	1.65 (1.33, 2.05)	<0.0001

eGFR: estimated glomerular filtration rate; APACHE II: Acute Physiology And Chronic Health Evaluation II; NI: Not Included. Forward censoring was applied and variables with p<0.2 in the univariate analysis were entered into the multivariate model.

Diagram D1 Example of the site-specific interventional algorithm, site 'Aarhus'

The Procalcitonin And Survival Study (PASS) Intervention Algorithm, Site: Aarhus

IMPORTANT: All patients shall (at least) receive antimicrobial therapy covering "standard-of-care", i.e. if any existing guidelines or evidence for antimicrobial treatment indicate/ contra-indicate surgical and/or antibiotic treatment, then the patient should be treated according to this. Indicated treatment should never be left out because of a possibly low procalcitonin (PCT).

All (except for the above standing situations) patients in the "PCT intervention" group must have treatment according to the present guidelines, including interventions when procalcitonin is ≥1,0 ng/ml and "Alert"[a].

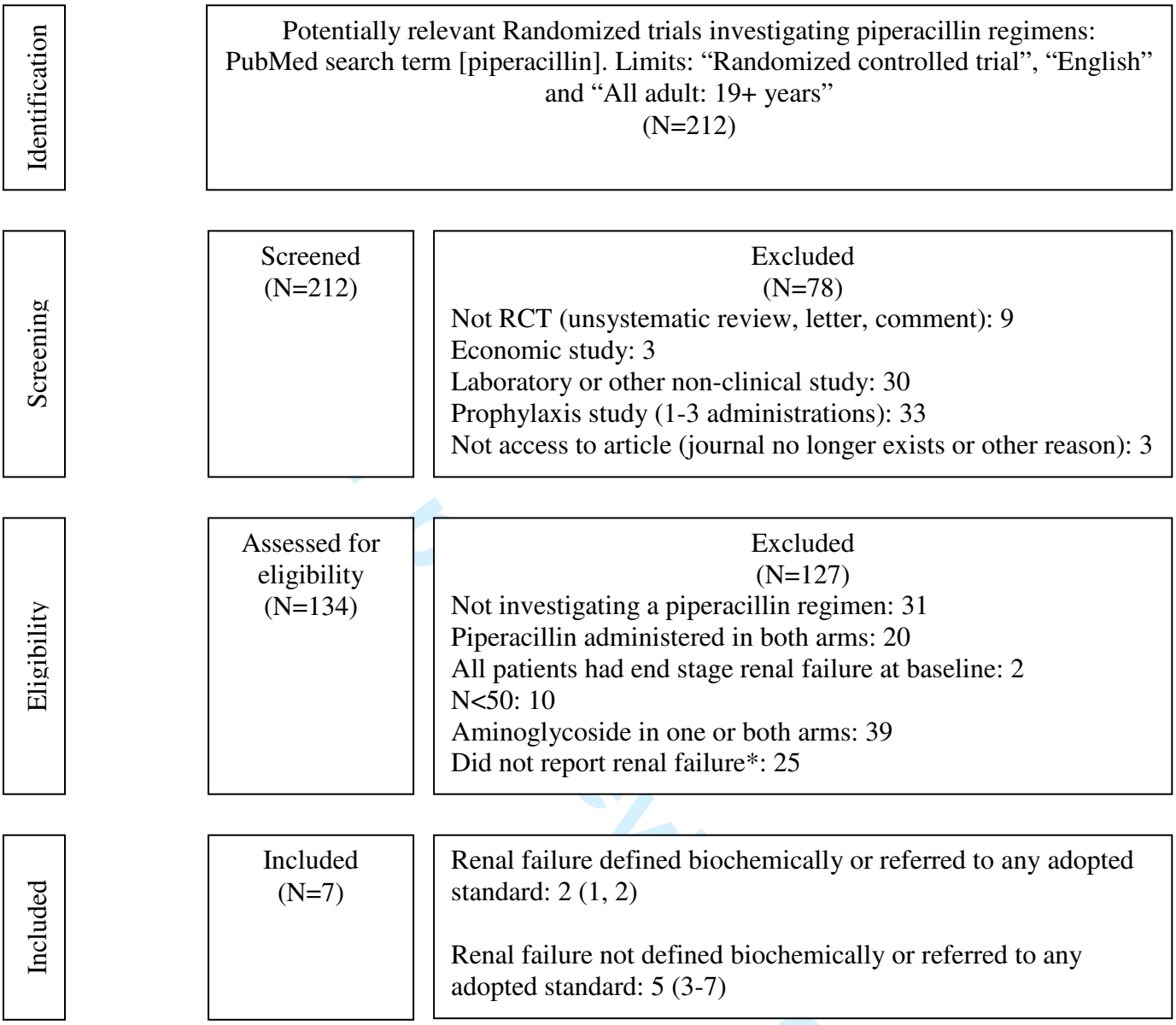
Patients are categorized daily according to the PASS intervention categories, on the basis on the present and the previous PCT measurement (displayed as "Alert" or "Non-Alert" in the website). In correspondence with every category, a PASS-intervention is displayed below. The treatment is, adjusted according to new and relevant microbiology that "explains" the clinical picture

CATEGORY 1	First PCT > 1,0 ng/ml, patient has not received antibiotics (≥1 DDD[b] within 72 h)
CATEGORY 2	A) First PCT ≥1,0 ng/ml, patient has received antibiotics (≥ DDD[b] within 72 h) or B) PCT "Alert" for 1 day after CAT 1,CAT 4 or CAT 5 has been started or C) PCT "Alert"** from "start-sample" till next morning
CATEGORY 3	A) First PCT ≥1,0 ng/ml, patient has received antibiotics (≥ DDD[b] within 72 h) and clinical suspicion of fungal infection or catheter related infection. or B) PCT "Alert" for 1 day after CAT 2 has been started
CATEGORY 4	A) Start PCT< 1,0 ng/ml or B) "Non-Alert" PCT, but ≥ 1,0 ng/ml. or C) PCT < 1,0 for 1-2 days
CATEGORY 5	PCT < 1,0 ng/ml for 3 or more days.

Action / Category	Diagnostics	Surgery	Antimicrobials[c]
CATEGORY 1	• Blood culture • Tracheal secretion • Urine culture • Culture from susp. source • Diagnostic imaging of susp. source	According to diagnostic imaging and clinical judgment	1. Cefuroxim 1500 mg x 3 i.v. or Ampicillin 1g x 4 / 2 g x 3 i.v. 2. Ciprofloxacin 400 mg x 2 i.v. 3. Consider: Metronidazol 500 mg x 2 i.v.
CATEGORY 2	• Blood culture • Tracheal secretion • Urine culture • Culture from susp. source • Diagnostic imaging of susp. source	According to diagnostic imaging and clinical judgment	1. Pip/Tazo[d] 4gx3 iv or Meropenem 1gx3 iv 2. Ciprofloxacin 400 mg x 2 i.v. 3. Metronidazol 500 mg x 2 i.v. 4. Consider fungal infection: Fluconazole i.v. and cath. inf: Vancomycin, dosage acc.to. Se-Vanco[e]
CATEGORY 3	• Blood culture • Tracheal secretion • Urine culture • Culture from susp. source • Diagnostic imaging of susp. source • Renewing oldest diagnostic imaging of susp. source	According to diagnostic imaging and clinical judgment	1. Pip/Tazo[d] 4gx3 iv or Meropenem 1gx3 iv 2. Ciprofloxacin 400 mg x 2 i.v. 3. Metronidazol 500 mg x 2 i.v. 4. Fluconazol 400 mg x 2 i.v. 5. Vancomycin, dosage acc.to. Se-Vanco[e]
CATEGORY 4	Nothing further	Standard-of-care approach	Continue present treatment
CATEGORY 5	Nothing further	Standard-of-care approach	Re-consider the indication for antibiotics (standard-of-care principle)

[a] 'Alert PCT' is defined as PCT-day1 ≥ PCT day 0 x 0.9. So a decrease in PCT from 11,2 ng/ ml to 10,5 ng/ ml is an "irrelevant decrease" and is defined as an "Alert" PCT. [b]DDD = Defined Daily Dosages). N.B.: The mentioned dosages are examples. Dosing regimen and frequency is prescribed according to the department guidelines (according to weight, kidney function, haemodialysis, Continuous dialysis etc.). [c]Antimicrobial spectrum covered can be broader than suggested (discretion of investigator). Administration of antimicrobials with a narrower spectrum on Alert-PCT days, should only take place when any antimicrobial treatment covering the suggested spectrum is contra-indicated and such a therapy should always be discussed and accepted by the coordinating centre. [d]Pip/Tazo: piperacillin/tazobactam. [e]Se-Vanco: serum-vancomycin measurements

Diagram D2:
Meta-analysis of randomized trials using piperacillin-containing regimens exploring renal failure



Results:

- In the initial identification phase, four ICU studies were found: They were excluded, since A) only a (non-defined) part of the patients received piperacillin(8), B) Both groups received piperacillin(9), C) one or both groups received aminoglycosides concomitantly(10, 11) .
- In the 7 (non-ICU) trials eventually included, 1592 episodes of therapy were observed.
- 21 cases of renal failure (not defined) occurred, corresponding to 1.3%.
- Hypothesizing, that the incidence of renal failure is 0.5% in non-piperacillin containing beta-lactam therapies, and aiming to find a risk increase to totally 1.5% (relative risk of 3.0), using conventional type I risk limit of 5% and a power of 80%, the sample size for such a trial investigating this should be approx. 3300 patients (non-ICU setting).
- In an ICU setting, the incidence of renal failure is often >20%. A trial of 1000 patients would be able to detect a risk increase to 28% (Relative risk:1.4) from e.g. piperacillin

*All articles were reviewed for this. Additionally, in adobe documents with the search option (those not scanned), a search was made in each pdf document with search terms: "renal", "kidney", "nephro", "creatinine" and "gfr". More than the noted 25 of the articles did not report renal failure, however, if they fulfilled one or more of the other exclusion criteria, they were excluded because of this.

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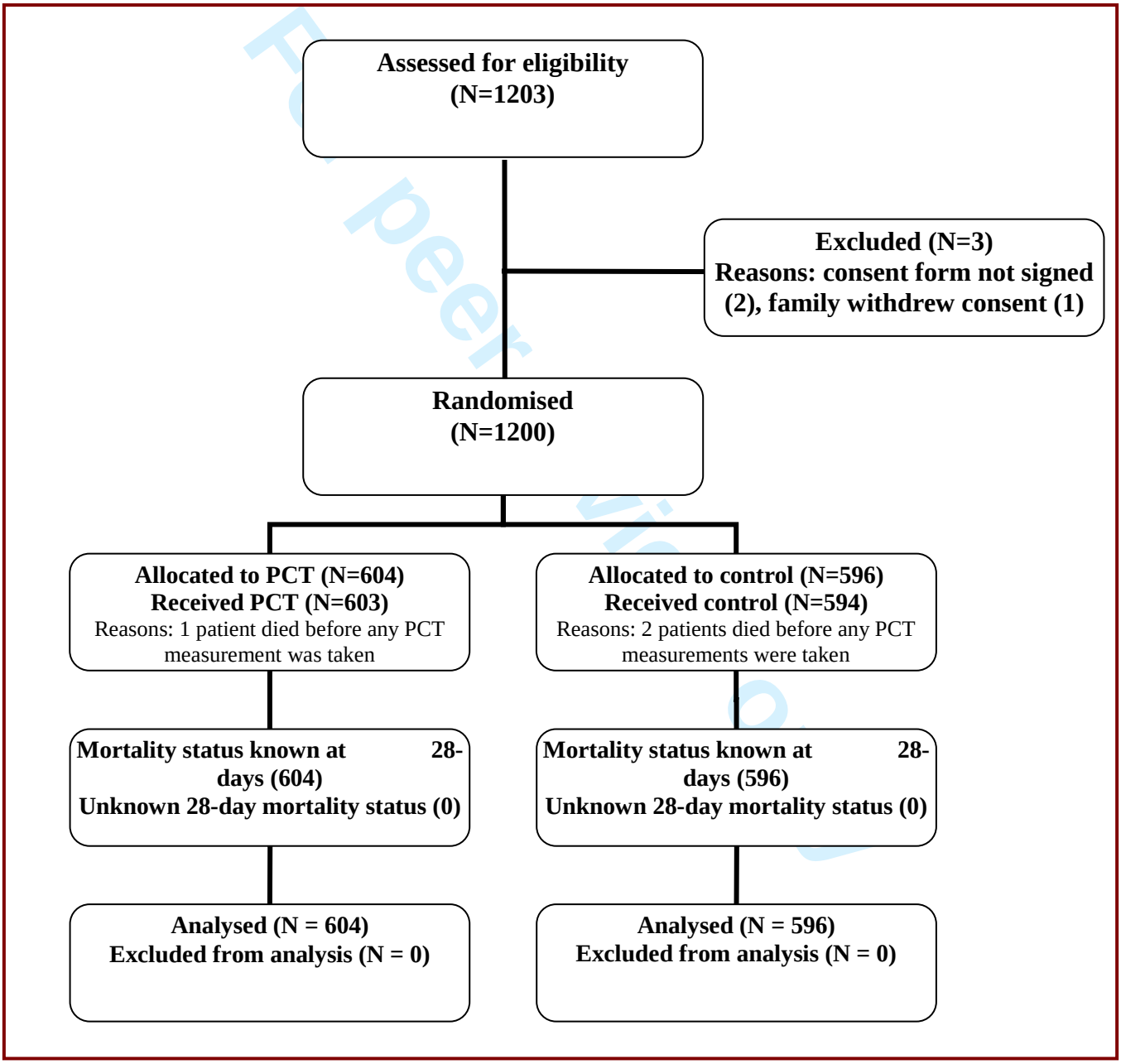
PASS-II 25th Aug 2010

Antibiotics and Renal Organ Failure – secondary endpoints from the Procalcitonin And Survival Study - analysis plan

1. Consort Flow Diagram (done in PASS-1)



Trial profile.

2. Baseline characteristics

Table 1: Baseline characteristics

	Standard-of-care-only n=596)	Procalcitonin-guided n=604)	Overall n=1200)
Age (Yr.) Median (IQR)	67 (58–75)	67 (58–76)	67 (58–76)
Male sex – no. (%)	333 (55·9%)	330 (54·6%)	663 (55·3%)
Body Mass Index – Median kg/m2 (IQR)	24·7 (22·0–27·8)	25·0 (22·5–28·7)	24·8 (22·2–27·9)
APACHE II Score - Median (IQR)	18 (13–24)	18 (13–25)	18 (13–24)
Surgical patient – no. (%)	260 (43·6)	227 (37·6)	487 (40·6)
Chronic co-morbidity* - no. (%)			
No chronic co-morbidities	102 (17·1)	123 (20·4)	225 (18·8)
1 chronic co-morbidities	279 (46·8)	257 (42·6)	536 (44·7)
2 chronic co-morbidities	173 (29·0)	171 (28·3)	344 (28·7)
≥3 chronic co-morbidities	42 (7·1)	53 (8·8)	95 (7·9)
Acute illness/reason for admittance to ICU – no. (%)			
Central nervous system incl. Unconsciousness	78 (13·1)	101 (16·7)	179 (14·9)
Respiratory failure	422 (70·8)	410 (67·9)	832 (69·3)
Circulatory failure	263 (44·1)	257 (42·6)	520 (43·3)
Gastro-intestinal disease	128 (21·5)	96 (15·9)	224 (18·7)
Renal disease	81 (13·6)	103 (17·1)	184 (15·3)
Post-operative complications	123 (20·6)	106 (17·6)	229 (19·1)
Trauma	113 (19·0)	106 (17·6)	219 (18·3)
Other	68 (11·4)	57 (9·4)	125 (10·4)
Indicators of severity			
Temperature, ^{0}C (median (IQR), n=1136)	37·3 (36·3–38·1)	37·4 (36·4–38·3)	37·3 (36·3–38·2)
Mean arterial pressure, mmHg (median (IQR) n=1195)	71 (60–84)	72 (63–85)	71 (62–84)
Heart frequency (median (IQR) n=1197)	100 (82–116)	100 (84–117)	100 (83–117)
Need for vasopressor/inotropic drug† (%, n=1200)	315 (52·9)	326 (53·4)	641 (53·4)
PaO2 /PaCO2 ratio (median (IQR), n=1178)	1·85 (1·27–2·62)	1·82 (1·29–2·53)	1·83 (1·28–2·59)
pH (median (IQR) n=1185)	7·29 (7·21–7·39)	7·29 (7·20–7·38)	7·29 (7·20–7·38)
Mechanical ventilation used (%, n=1200)	401 (67·3%)	401 (66·4%)	802 (66·8%)
Creatinine µmol/lL (median (IQR) n=1167)	119 (78–197)	119 (75–208)	119 (76–202)
Dialysis required (%, n=1200)	88 (14·8%)	86 (14·2%)	174 (14·5)
Bilirubin, µmol/L (median (IQR) n=1109)	10 (6–17)	10 (5–18)	10 (5–17)
Infection, clinical assessment ‡ – no. (%)			
No infection	118 (19·8)	86 (14·2)	204 (17·0)
Localized infection or Sepsis	266 (44·6)	271 (44·9)	537 (44·8)
Severe sepsis/ septic Shock	212 (35·6)	247 (40·9)	459 (38·3)
Site of infection § – no. (%)			
CNS	12 (2·0)	35 (5·8)	47 (3·9)
Respiratory	292 (50·0)	324 (53·6)	616 (51·3)
Gastrointestinal	149 (25·0)	145 (24·0)	294 (24·5)
Urinary	28 (4·7)	42 (7·0)	70 (5·8)
Other	52 (8·7)	41 (6·8)	93 (7·8)
Biomarkers			
Alert-PCT || – no. (%)	279 (47·0)	312 (51·7)	591 (49·4)
Leukocytes, x10^{9} – median (IQR)	13·0 (8·8–18·1)	12·4 (8·0–18·1)	12·8 (8·4–18·1)
C-reactive protein, mg/L – median (IQR)	152 (54–266)	161 (56–271)	157 (56–271)

Interquartile range (IQR). Acute Physiology and Chronic Health Evaluation II score (APACHE II) ranges from 0 to 71. *Chronic co-morbidity: Earlier diagnosed via hospital admission: heart failure, lung disease, cancer, diabetes, alcohol abuse, chronic infection, neurological disease, renal diseases, liver disease, gastro-intestinal disease, autoimmune disease, cancer and psychiatric disorders. Acute illness: persons can have several. 'Other' includes liver disease, haemorrhage, haematological disease and poisoning. †Vasopressors/inotropic drugs are considered to be epinephrine, nor-epinephrine, dopamine and dobutamine. ‡ Infections were rated according to the ACCP/SCCM definitions; investigators were trained in using them. § Site of infection: patients can have more than one. ||Alert-PCT: Procalcitonin-level not decreasing by at least 10% from the previous day and above 1·0 ng/ml. If only one measurement is available: Absolute procalcitonin-level above 1·0 ng/ml.

Table 1. Baseline characteristics of the study participants.

Table 2: Follow up characteristics

Follow up measurement	Control group (N=596)	PCT-guided group (N=604)	Overall (n=1200)
Patients followed and alive for 28 days (N., %)			
Patients followed for 28 days (incl. those who died in the first 28 days) (N., %)			
Status at 28 days (n =): Alive Dead			
Days spent in ICU Median (IQR) (as in PASS-I)			
Days spent in Danish hospital within 28 days Median (IQR)			
Patients with a complete 28 day follow up for respiratory failure (mech. Vent., PaO2 and FiO2)			
Days followed within 28 days for respiratory failure (mech. Vent, PaO2 and FiO2) of total days in trial ((denom. = 604 x 28) this can be drawn from the admission list in combination w. database)			
Patients with 28 day follow up for renal failure (dialysis – same as prev.)			
Days followed within 28 days for renal failure (dialysis) of total days in trial (denominator = 604 x 28 and 596 x 28 days) (same as prev.)			
Patients with 28 day follow up for renal failure (eGFR)			
Days followed within 28 days for renal failure (eGFR) of total days in trial (denominator = 604 x 28 and 596 x 28 days)			
Patients with 28 day follow up for Platelets			
Patients with 28 day follow up for Bilirubin			
Patients with 28 day follow up for antibiotic consumption			

n*s refers to the total number of patients who had follow up for 28 days.
28-day follow up is: Follow up until death within 28 days OR until day 28. For respiratory failure follow up is done for all ICU admissions. For renal failure, follow up is done for all dialysis treatment (ICU+other dialysis competent hospital units) and for all creatinine and carbamide measurements performed within 28 days (ICU + non-ICU admissions). For platelets and bilirubin, follow up is done for all measurements performed within 28 days (ICU + non-ICU admissions)

STRATIFICATION (*S) / test for interaction: (regarding the below analyses in Section 2 + 3)

1. **Age (limit initially 65 y, if significant interaction, more age groups**
2. **APACHE II score (limit initially 20, if significant interaction, more APACHE II groups,**
3. **Site 1-9.**
4. **Severe Sepsis/septic Shock vs. Milder or No infection at Baseline**
5. **Calendar date of inclusion into PASS. Recruited: 9th Jan 2006 – 31st December 2007 (~430 patients) vs. 1st of Jan 2008 – 2nd of June 2009 (~770 patients).**
6. **Surgical patient / medical patient [Surgical = All patients with mark in Baseline "B6", or "B12" or marked "Yes" in "L"]**
7. **Gender**

SECTION 2. Exposure – Antibiotic usage

Follow up: All patients were followed up regarding antibiotic consumption: 1) In the ICU in the primary PASS-CRF, 2) All ICU-surviving patients, not staying in the ICU for 28 days, were followed up for antibiotic consumption in the non-ICU, they were discharged to after ICU.

General: The aims of these analyses are to investigate the impact of performing PCT-guided empiric antibiotic interventions according to a progressive algorithm on the consumption of antibiotics. This is to be illustrated by analyses exploring 1) spectrum, 2) quantity and 3) duration of therapy in the two arms.

The aim is:

a) To investigate the difference in exposure in general to antibiotics in the two arms of the PASS trial and more specifically to broad-spectrum antibiotics.

This is done in the following analyses (PCT vs. Control):

1) The total number of days within the 28 day follow-up period with any antibiotic treatment (or proportion of follow-up time): [Not done Yet]
2) The total consumption of any antibiotic in weight (grams within 28 days) [Not done Yet]
3) The total consumption per ICU day of any antimicrobial [DONE]
4) The total consumption of betalactam drugs active against most Extended Spectrum Beta-lactamases and wild-type Pseudomonas aeruginosa (a. Meropenem and other pseudomonas active carbapenems, OR b. Piperacillin/tazobactam OR c. 4.generation Cephalosporins). [or proptortion of days in these treatments] [Not done Yet]
5) The total no. of days within the 28-day follow up period with treatment with any flour-quinolone (ciprofloxacin, moxifloxacin and others) [or proptortion of days in these treatments] [Not done Yet]
6) The total no. of days within the 28-day follow up period with treatment with any glycopeptide (Vancomycin, Teicoplanin) [or proptortion of days in these treatments] [Not done Yet]
7) The total no. of days within the 28-day follow up period with treatment with fluconazole [or proportion of days in these treatments] [Not done Yet]

Consumption of antimicrobials in the intensive care unit				
Length of antimicrobial treatment in ICU, days (median, IQR)	4 (3– 10)	6 (3– 11)	-	0·001
Quantity of antimicrobials administered per ICU day (g) (median, IQR)	6·7g (4·5g– 12·5g)	8·6g (5·3g– 13·7g)	-	<0·001
Number (%) ICU days spent with at least three antimicrobials	2721 (57·7%)	3570 (65·5%)	-7·9% (-9·7%– -6·0%)	0·002

*Counted from the time of sampling. Only samples later to become positive. Cultures with coagulase negative staphylococci, corynebacteria and propionebacteria are not included. † Including localised infection, mild sepsis, severe sepsis and septic shock. p-values for the number of days spent with each factor were generated by testing the proportion of intensive care days spent with each factor using non-parametric tests. ICU: Intensive care unit

Table 3. Antibiotic consumption

Admission time within 28 days

1. Number of days admitted to hospital within 28 days after recruitment. Median + IQR. (PCT vs. Control)

Subgroup Analysis: Total use of Antimicrobial chemotherapy

1. Total antibiotic prescription days (all AMCs received, where all AMCs are weighted equally and summed per day, e.g.:→ possible to have e.g. 30 prescription days in 10 days ICU)

Table 3: Number of AMCs received per day (over all days)

	PCT-arm	Control-arm	P-value
AMC total (N,. %)			
Recruited 09/01/06 – 31/12/07 Recruited 01/01/08 – 02/06/09			
Age <65 years Age ≥65 years			
APACHE II <20 APACHE II ≥20			
Bispebjerg Gentofte Glostrup Herlev Hillerød Hvidovre Roskilde Skejby Århus			
Severe Sepsis or septic shock at BL Milder or no infection at BL			
Surgical patient Non-surgical patient			
Gender			

MICROBIOLOGY

Follow up: All patients were followed up via the electronic registers at the microbiologic depts., who service the PASS-ICU´s regarding all microbiologic samples performed from baseline and until 28 days after. Data have been merged in the PASS-database.

Table 4: Number of culture samples performed within 28-days from randomisation [Not done Yet – JU handles this]

Intervention		PCT arm N =	Control Arm N =	P-value
Microbiology:	**N., (%)**			
Blood Cultures	N. Yes, (%)			
Urine Cultures	N. Yes, (%)			
Airway Cultures	N. Yes, (%)			
Samples from other foci	N. Yes, (%)			

SECTION 3a: Estimating the degree of Organ Failure (OF)

Follow up: All patients were followed up regarding respiratory failure (mech. Vent + physiologic parameters) and renal failure at 1) the PASS-ICU where the patient was recruited in the primary PASS-crf, 2) regarding mech. Ventilation and physiologic parameters and renal failure at any other PASS-ICU within the 28 day period (when patients were discharged to such an ICU, 3) in the case that a patient was discharged within the 28 day period to a non-PASS ICU (seldom), follow up was made for mech. Vent. and physiologic parameters and renal failure in hospitals "Rigshospitalet" and "Bispebjerg", since only very few ICU days were spent at any other ICU within the 28 day period (48 days of approx 9900 days = approx 0.5%).

The purpose of these analyses is to explore in detail, the quantity of the occurrence of secondary endpoints in the PASS-trial, especially respiratory organ failure and renal organ failure.

Genuine hypothesis: High usage of broad spectrum antibiotics as used in the PASS trial, results in substantially reduced organ function (respiratory, renal and liver) and compromised coagulation and a likewise substantially increased time with manifest organ failure as defined clinically (need for organ support) AND biochemically/fysiologically (measured objective parameters).

NB: Analyzes are summarized in the table 5 below

time)

A. Renal Failure:

a. Median/ Mean eGFR for day1 – day10
b. Median/ Mean eGFR for day11 – day28
c. Median/Mean eGFR for day1 – day28 (a+b) [eGFR on days in columns in a figure and AUC for the columns]
d. Median/Mean Carbamide for day1- day10
e. Median/ Mean Carbamide for day11 – day28
f. Median/Mean Carbamide for day1 – day28 (a+b) [Carbamide level on days in columns in a figure and AUC for the columns]
g. Median/Mean Platelet count for day 1-28 [[platelet on days in columns in a figure and AUC for the columns]
h. Median/Mean Bilirubin [Bilirubin on days in columns in a figure and AUC for the columns]
i. No. of days within 28 days with eGFR < 60 ml/min/1.73 m2
j. No. of days within day1 – day10 with eGFR < 60 ml/min/1.73 m2
k. No. of days within day1 – day10 with dialysis
l. No. of days within day11 – day28 with dialysis
m. No. of days within day1 – day28 with dialysis

C + F+ G + H are all part of one figure with 4 panels.

Explanations: A: Dialysis:

Patients are categorized on days with ND or NA as dialysis=0, since this means patient has been discharged to home. All admissions within 28 days have been drawn from the central hospital register (Green System) and all admissions at dialysis capable departments have been followed up with dialysis.

B: eGFR:

In the ICU, patients are categorized with a new eGFR every day (done in PASS).

Patients are categorized on the basis of their status of eGFR on the last day of ICU. This status is kept until a creatinine measurement is done (on which day the status is changed to a new eGFR). This status is then kept until the next time creatinine is measured – and so forth. In this way every day from 1 – 28 is given an eGFR status.

In summary, the same principle is used: From day 1, the first time a creatinine is measured, a eGFR is calculated. Next time the patient has a creatinine measurement, the patient is re-categorized with a new eGFR. That eGFR is kept until the next creatinine measurement etc.

Table 5. Prevalence and duration of organ failure and other severe disturbances (PCT vs. Control)

	PCT arm (n =)	Control Arm (n =)	P-value
Kidney Failure mL/min/1.73 m^2 (N. days, % of total days): Normal: GFR > 90 Mildly impaired: 60–89 Moderately/severely impaired: GFR <60			
Kidney Failure Median/ Mean eGFR for day1 – day10			
Kidney Failure Median/ Mean eGFR for day11 – day28			
Kidney Failure Median/Mean eGFR for day1 – day28 (a+b)			
Kidney Failure Median/Mean Carbamide for day1- day10			
Kidney Failure Median/ Mean Carbamide for day11 – day28			
Kidney Failure No. of days within 28 days with eGFR < 60 ml/min/1.73 m2			
Kidney Failure No. of days within day1 – day10 with eGFR < 60 ml/min/1.73 m2			
Kidney Failure No. of days within day1 – day10 with dialysis			
Kidney Failure No. of days within day11 – day28 with dialysis			
Kidney Failure No. of days within day1 – day28 with dialysis			

Table with summarized analyses.

SECTION 3b: Attempting to explain the reason for organ failure (if OF is confirmed in section 3a)

Antimicrobial toxic explanation

Genuine hypotheses:

1) High Exposure (at least 5 or at least 10 days) to a certain combination of antibiotics (Pip/Tazo+Cipro OR Meropenem + Cipro OR Pip/Tazo + Vanco OR Meropenem + Vanco) causes OF

For 2-6: Estimate accumulated risk for day 1, 2, 3 etc. separately in both PCT group and control group.

2) Treatment for more than 4 days with Pip/Tazo causes OF (also 10 days)
3) Treatment for more than 4 days with Ciprofloxacin causes OF (also 10 days)
4) Treatment for more than 4 days with Meropenem causes OF (also 10 days)
5) Treatment for more than 4 days with Vancomycin causes OF (also 10 days)
6) Treatment for more than 4 days with Cefuroxim causes OF (also 10 days)

For the below analyses two composite endpoints are used for the Pulmonary/renal OF:

1) **Organ failure endpoint A**: Clinical Organ Failure judgment: Endpoint=1 for any day with dialysis. If both are present, Endpoint=2. Results are presented as “Clinical Organ Failure Days”
2) **Organ failure endpoint B:** Objective Organ failure measures: Endpoint =1 for any day with eGFR <30, repeated with <60 ml/min/1,73 m2. “Objective Organ Failure Days”

Analyses:

A. Objective Organ failure endpoint:

As above, 1) – 6).

1) Analyze the median “Objective Organ Failure Days” to occur from “P-T treatment day 5” until 10 days later (counting endpoints for next 10 days). Censor at death.
2) Analyze the median “Objective Organ Failure Days” to occur from “Meropenem treatment day 5” until 10 days later (counting endpoints for next 10 days). Censor at death

B. Multiple Effects models:

Regarding renal dysfunction: Analyze renal recovery in eGFR progression per day on different drugs day 1-10 (Meropenem / Piperacillin-tazobactam / Ciprofloxacin / Cefuroxim), control for other known predictors of renal failure. Additionally after discontinuation of these drugs.

Sensitivity analyzes:

Cox or Logistic Regression ?

Endpoint: Binary endpoint. To be defined according to the median number of organ failure days within 10 days after exposure for 5 days.

Endpoint 1a: [>median number of “clinical organ failure days”]

Endpoint 1b: [>median number of “clinical organ failure days”+2 days]

Endpoint 2a: [>median number of “objective organ failure days”]

Endpoint 2b: [>median number of “objective organ failure days”+2 days]

Risk variables to be entered:

a. Treatment for >=4 days with Pip/tazo

b. Treatment for >=4 days with Meropenem

c. Treatment for >=4 days with Ciprofloxacin

d. Treatment for >=4 days with Vancomycin

e. Treatment for >=4 days with Pip/tazo + Ciprofloxacin (all 4 days)

f. Treatment for >=4 days with Meropenem + Ciprofloxacin (all 4 days)

g. Treatment for >=4 days with Pip/tazo + Vancomycin (all 4 days)

h. Treatment for >=4 days with Meropenem + Ciprofloxacin (all 4 days)

i. Treatment for >=4 days with Meropenem + Vancomycin (all 4 days)

j. APACHE II >=20

k. Age >=65

l. Surgical patient

m. Severe sepsis/septic shock

NB: Treatment count start days 1 – 13 (so 5 days complete on day 5 – 18).

Patients with pauses in the administration of >=1 day → exclude

Only count the first administration

Endpoints:

“Clinical Organ Failure Days” and “Objective Organ Failure Days” both as defined above

→Transformed to Binary endpoint:

Endpoint 1a: [>median number of “clinical organ failure days”]

Endpoint 2a: [>median number of “objective organ failure days”]

(as above in the sensitivity analysis)

PASS-II, organ failure – authors,

Forfattere

Chip: JU+JDL+LRN

KMA Hvh/Diacenter: BEL

Glostrup: Mulige: Asger, Anne, Ditte

Hvh: Mulige: Peder C, Jesper, Morten

Herlev: Mulige: Peter, Hamid, Tina

Gentofte: Mulige: Thomas, Katrin

Hillerød: Mulige: Morten, Lars, Kristian A?

Roskilde: Mulige : Niels-Erik

Århus: Mulige: Kim + Mads

Skejby: Mulige: Paul

Protocol

A randomised, single-blinded, multicentre trial to investigate if clinical management guided by daily standardised Procalcitonin measurements can reduce the mortality in critically ill patients

The Procalcitonin and Survival Study (PASS)

Version of protocol: 3.1

Date: December 2006

Intensive Care Units from many University Hospitals all over Denmark will participate:

Sponsor: Scientific:
Copenhagen HIV Programme (CHIP) 044, Hvidovre University Hospital, Denmark
: Economic: Danish Research Council (Danish State) and other independent research foundations

Protocol co-ordinator

Jens-Ulrik Stæhr Jensen
H:S Hvidovre University Hospital
DK - 2650 Hvidovre
Denmark
Phone: +45 36 32 33 07
Fax: +45 36 47 33 40
E-mail: koordinator@pass-studiet.dk

INVESTIGATOR PROTOCOL AGREEMENT PAGE

THIS AGREEMENT IS EQUIVALENT TO A "SIGNED PROTOCOL"

The PASS Trial

Name and qualifications of investigator:

Name of Investigator: _____

Post held: _____

Clinical Centre: _____

I agree:

- to assume responsibility for the proper conduct of the PASS Trial at this site.
- to conduct the trial in compliance with this protocol, any future amendments, and with any other trial conduct procedures provided.
- not to implement any deviations from or changes to the protocol without agreement from the sponsor and prior review and written approval from the Independent Ethics Committee (IEC), except where necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the trial (where permitted by all applicable regulatory requirements).
- that I am thoroughly familiar with the appropriate use of the Procalcitonin test and the interpretation of the test results, as described in this protocol, and any other information provided by the manufacturer of the test and by the PASS Coordinating centre.
- that I am aware of, and will comply with, "Good Clinical Practice'' (ICH-GCP Guideline (CPMP/ICH/135/95, Directive 2001/20/EC)) and all applicable regulatory requirements.
- to ensure that all persons assisting me with the trial are adequately informed about the Procalcitonin test and interpretation and of their trial-related duties and functions as described in the protocol.

_____ _____

Signature of investigator Date

One signed copy each to be held by the Investigator and PASS Co-ordinating centre.

15/10/2007

TABLE OF CONTENTS

A randomised, single blinded, multicentre trial to evaluate whether daily Procalcitonin measurements and immediate diagnostic and therapeutic response on abnormal values and day-to-day changes can reduce the mortality of critically ill patients in the Intensive Care Unit.

The Procalcitonin And Survival Study (PASS)

PROTOCOL SUMMARY

Inclusion:

Fulfilment of all of the following three criteria:

1 Male or female, aged ≥ 18 years of age.

2 Admitted to the participating intensive care units (ICU) at following hospitals: Hvidovre Hospital; Bispebjerg Hospital; Herlev Hospital; Glostrup Hospital; Gentofte Hospital; Hillerød Hospital; Roskilde Hospital; Århus University Hospital, Århus; Århus University Hospital, Skejby.

3 1) Ability to understand and provide written informed consent to participate in this trial,

or

2) Ability to understand and provide oral informed consent in presence of at least one impartial witness who should sign and personally date the consent form

or

3) The subjects legally acceptable representative can understand and provide written informed consent if the subject is not capable of this because of the present mental or physical condition of the subject.

Exclusion:

A subject will **NOT** be eligible for inclusion in this trial if any of the following criteria apply:

1. Subjects with known hyper-bilirubinaemia (>0.4 mg/ ml) or hypertriglyceridaemia (>10 g/l) since this can interfere with measurements. If subjects with unknown status on these points are included and have PCT measurements, the measuring-equipment will detect these conditions.

2. Subjects suffering from a blood disorder, where daily sampling of 7 ml of blood for maximally 28 days (210 ml distributed on 28 days) will be an inconvenience or a potential risk, which could compromise the safety of the subject.

3. Subjects who are pregnant or breast feeding

The *a priori* probability of surviving with the normal recommended diagnostics and treatment with the presently available means to detect infections and on the other hand the normal diagnostics and treatment together with daily Procalcitonin measurements and prompt clinical reaction should be equal.

Randomisation:

Two arms (1:1), n = 500 per arm:

Arm 1: Normal recommended diagnostics and treatment of infections in the intensive care unit (standard of care)

Arm 2: Normal recommended diagnostics and treatment of infections in the intensive care unit (standard of care) **and** Procalcitonin guided diagnostics and treatment of infection

Primary Trial Objective: To address whether daily Procalcitonin measurements and immediate diagnostic and therapeutic response on abnormal values and day-to-day changes can reduce the mortality of critically ill patients in the ICU.

Trial registration days: Intensive Care Unit admission day, running routine registration of examinations and blood tests, day of discharge or death, day 28 after admission, day 60, 90, 120 and 180 after discharge.

Data collection: The data collection will be simple and performed real time via fax.

1 TRIAL BACKGROUND AND RATIONALE

1.1 Background

1.1.1 *Sepsis and mortality in the Intensive Care Unit*

Sepsis remains a major cause of mortality in critically ill patients admitted to the Intensive Care Unit (ICU) [1-2]. All-cause mortality during ICU admission ranges from 12.1% in non-infected patients to 43.9% in infected patients[3]. Patients who are discharged to other departments and later to their own home or an institution for rehabilitation, continue to have a high mortality (additionally 10-20%) for 20-30 days after ICU discharge[4-7]. Different explanations for this have been proposed. Among the most important are:

1) During ICU admission it becomes clear that further treatment lacks perspective for the patient (often chronical organ diseases and cancer diseases) and the patient is therefore discharged to the relevant department when discharge from the ICU is possible.
2) After discharge from the ICU the physical condition of the patient deteriorates because of a severe disease with a dismal prognosis and it is decided together with the patient and relatives that the patient should not be admitted to the ICU again.
3) Critically ill patients often have an immunological incompetence and therefore these patients are susceptible to serious infections. Additionally these infections often have an atypical course and thereby a delayed diagnosis. This immunological incompetence prevails some time after discharge from the ICU why the patient remains susceptible to infections for this period of time. There is a grave risk that these serious infections with an atypical course can be diagnosed late in the course and cause an increased risk of mortality for critically ill patients.

1.1.2 *Procalcitonin and bacterial infections*

In 1993 Assicot et al. reported that a high level of serum-Procalcitonin (PCT) was closely related to bacterial infection and seemingly correlated to the severity of the infection[8]. This finding has since been ascertained in many studies demonstrating high levels (2.0 ng/ml-50.0 ng/ml (-1500 ng/ml)) of PCT in patients with systemic bacterial infection, while low levels have consistently been found in patients with localised bacterial infections and viral infections[9-16]. Others have shown low PCT levels (and seldom up till maximally 3.0 ng/ml) in non-infected patients following surgery, trauma and myocardial infarction[10, 17-21]. Sensitivity and specificity for sepsis when PCT levels are above 5.0 ng/ml have been estimated to 80-90 % and 85-100%, respectively, in the largest of these studies.
The PCT level starts decreasing within 24 h after surgery, trauma and myocardial infarction in non-infected patients in contrast to the C-reactive protein, which has a peak level 36-72 h after these events[10-17-21].

Consequently, bacterial infection is suspected if PCT is increasing 24 h after surgery, trauma or myocardial infarction.

1.1.3 *Procalcitonin kinetics, biochemistry and cellular biology*

PCT is a 13 kDa, 116 amino acid polypeptide, initially described as a pro-hormone of Calcitonin, a

hormone in the calcium metabolism, which is produced in the medullary C-cells in the thyroid gland[22-24]. Recent studies have shown that the PCT variant, which is related to infection is produced in other tissues (liver, kidney, muscle, fat)[25-27]

Kinetic studies with healthy humans and baboons have shown a rapid release of PCT within 2-6 hours after injection of bacteria or bacterial endotoxin. This time to release is significantly shorter than that of C-reactive protein (8-24 h). The plasma half life of PCT is approximately 24 h. PCT measurements in healthy, uninfected volunteers has been shown very low levels (<0.05 ng/ml)[10,28-29].

1.1.4 *Procalcitonin-guided treatment and reduction in the use of antimicrobial agents*

A recent study has demonstrated a reduced use of antimicrobial agents in patients with lower respiratory tract symptoms, when the treatment was guided by the initial PCT level[30].

1.1.5 *Procalcitonin and risk of mortality*

We have shown that a PCT increase after reaching a level of 1.0 ng/ml is an independent predictor of mortality in critically ill patients. Patients who did not reach a PCT level above 1.0 ng/ml had an all cause mortality risk of 4.7% while admitted in the ICU, compared to an all cause mortality of 19.1% for the whole population of ICU patients. Patients who reached a PCT value above 1.0 ng/ml who had a decreasing PCT the next day had a mortality risk of 18.9%, but patients who had an increasing PCT level after reaching 1.0 ng/ml had a mortality risk of 32.7%. This increase in mortality risk was significant for the entire follow-up period of 90 days[31].

The mortality risk increased for every day the PCT increased. Taking in mind the close relation between PCT levels and bacterial infection, a large part of this mortality increase is (when PCT is increasing), to the best of the existing knowledge, attributable to uncontrolled bacterial infections. This is supported by the findings of the European Sepsis Group[3].

The rapid release of PCT to the blood stream (2-6 h), when infection is progressing, makes acute detection of ongoing serious infection possible, hereby potentially reducing mortality in critically ill patients if treatment is guided acutely by PCT measurements.

1.2 Rationale - summary

Sepsis and complications to sepsis are major causes of mortality in critically ill patients[1-2]. Rapid treatment of sepsis is of crucial importance for survival of patients. In the ICU, the infectious status of the patient is often difficult to assess because symptoms cannot be expressed (unconscious or sedated patients) and signs may present atypically because of immunologic incompetence and masking by the drugs given and thermo-influencing-therapy, i.e. dialysis. Biological and biochemical markers of inflammation (WBC, C-reactive protein) may often be influenced by other parameters than infection, such as: trauma, surgery, other types of inflammation such as rheumatoid diseases (C-reactive protein) and gluco-corticosteroid treatment (WBC), and may be unacceptably slowly released after progression of an infection[32-33]. At the same time, lack of a relevant antimicrobial therapy in an early course of infection may be fatal for the patient.

For these reasons, in the clinical setting, it is often necessary to initiate or adjust antimicrobial therapy on an unsure ground and the relevant therapy may in some situations be delayed for important hours or even days. Specific and rapid markers of bacterial infection have been sought for use in the ICU. Mortality in critically ill patients increases gravely when Procalcitonin levels increase from day to day[31]. Low PCT levels have been shown to effectively rule out sepsis[12].

However, no randomised controlled trials have been conducted to show if mortality in critically ill patients can be reduced by using a strategy of daily standardised Procalcitonin measurements as an early detector of serious bacterial infection. Therefore evidence is presently not sufficient to introduce daily consecutive Procalcitonin measurements to guide the diagnostic and therapeutic management of patients admitted to the ICU .

The rationale for this trial is to assess the ability of daily Procalcitonin measurements to reduce the mortality of critically ill patients.

1.3 Procalcitonin analysing methods

There are four commercially available analysing methods for measuring blood levels of Procalcitonin, one semi-quantitative and three quantitative. Two of these are described below, the oldest and most used test, *LUMITEST ® BRAHMS /BRAHMS PCT LIA,* and a newer fully automated test with a higher sensitivity, *KRYPTOR® PCT BRAHMS.* KRYPTOR® PCT BRAHMS will be used for all Procalcitonin analyses in this study[34].

1.3.1 LUMITEST ® BRAHMS /BRAHMS PCT LIA

The oldest and so far most used quantitative test is LUMITEST ® BRAHMS /BRAHMS PCT LIA. Analysis is made by a ”sandwich” luminiscens immuno-assay with an anti-catacalcin coated tube:
Anti-**Catacalcin** binds catacalcin in the patient sample and is hereby immobilised (catacalcin could otherwise interfere with the analysis).
Anti-**Calcitonin** antibody is marked with a luminescent a*cridin*-derivative.
H_2O_2 and NaOH are added and these react with the *acridin*-derivative which leads to the formation of *acridon* and this process is accompanied by transmission of light. The quantity of this light is proportional to the Procalcitonin concentration in the sample.
We have found a coefficient of variation (CV) in the measuring interval between 0.1 ng/ml-1.0 ng/ml of 0.09-0.83 for this test. At PCT levels above 1.0 ng/ml, we found CV´s of 0.008-0.065 (range)[37].
The manufacturer claims a *functional assay sensitivity* (CV<0.2) of 0.3 ng/ml.

1.3.2 KRYPTOR® PCT BRAHMS

A new, and according to the manufacturer, more precise assay is the fully automated KRYPTOR® PCT BRAHMS. Procalcitonin is analysed using the analysing machine KRYPTOR® and fluids and utensils from the company BRAHMS diagnostica, Berlin. KRYPTOR® uses

TRACE technology (Time Resolved Amplified Cryptate Emission), which is a non-radiating transmission of energy. The transmission happens between two flourescent compounds: Europium Cryptate (donor) and XL665 (acceptor). While the antigen-antibody complex is formed, a signal is measured.

The functional assay sensitivity (CV< 0.2) is according to the manufacturer 0.06 ng/ml for the KRYPTOR ® test. In the relevant clinical interval (which has not quite been defined yet) the CV is 0.02-0.03 (product information).

- Studies concerning Procalcitonin have so far mainly been using LUMITEST ® BRAHMS /BRAHMS PCT LIA.

1.4 Rationale for a 24 h interval between blood sampling

Several studies have shown a half-life of Procalcitonin of 20-30 hours and Procalcitonin levels increase 2-6 h after bacterial products are presented in the blood stream [10,28-29, 35]. An important exception to this is patients suffering from severe uraemia, where the Procalcitonin half-life is prolonged, but it has been demonstrated, that Procalcitonin is removed by dialysis[35]. Studies concerning Procalcitonin and surgery have shown, that the Procalcitonin blood level is on a decreasing curve 24 h after major thoracic and abdominal surgery, except in infected patients[17-21]. In conclusion, a Procalcitonin level which is increasing 24 h after a therapy shift or after surgery suggests progression of infection.

1.5 Procalcitonin and immuno-compromised patients

Markers and mediators of inflammation and infection are often dependent on a functioning immune system, which is able to produce the substance measured, e.g. WBC, TNF, different interleukins[10,15,16, 36]. It has been established that Procalcitonin is not dependent on blood cells and their mediators, and Procalcitonin is mainly produced by tissues like liver, kidney, muscle and fat[25-28]. In concordance with this, studies investigating Procalcitonin in neutropenic patients have found results comparable to those for immuno-competent patients[36-41]. A few studies regarding neutropenic patients that compared PCT levels to positive blood cultures have found a low sensitivity of the test for bacteriemia, but these studies lack clear definitions of virulence of different micro-organisms (e.g. Coagulase negative staphylococci vs. Gram negative rods) in their study designs[40].

1.6 Studies on Procalcitonin biology and bacterial infection

1.6.1 In vitro and animal studies

In vitro studies have shown Procalcitonin to be an inducer of albumin synthesis in rat liver tissue measured on mRNA and protein synthesis. This was found to be opposite to TNFα and IL-6, these substances lowering albumin synthesis[42]. In a study of sepsis in baboons, low PCT was

found in non-infected subjects and high PCT in infected subjects, and PCT blood levels started increasing after 2 hours[10]. In another baboon model Procalcitonin incompetence was shown in an anhepatic subject[28].
In a study of burn wound and Pseudomonas aeruginosa septicaemia in rats, a high correlation between endotoxin levels and PCT in blood was found[43].

1.6.2 Human observational studies

Most of the present knowledge on Procalcitonin has been established by observational studies. Key-references are mentioned in paragraph 1.1 and 1.2

1.6.3 Clinical trials

Only few Randomized Controlled Trials regarding PCT-guided treatment have so far been published, one of special interest has used PCT-guided treatment (n=119+124)and has assessed the ability of this clinical strategy to reduce use of antimicrobial therapy in patients with suspected lower respiratory tract infection. A Relative Risk of 0.49 [95% CI 0.44-0.55] for antibiotic exposure was demonstrated, without any significant difference in culture growth from patient samples, quality of life, mortality, inflammatory parameters (temperature, C-reactive protein, WBC), number of days admitted and need for stay in intensive care unit. The study was designed to detect a 30 % difference with 95% stringency. However some of the mentioned endpoints do not occur in all patients, and in these cases (mortality, need for stay in ICU) it may be false to conclude, that there is no difference between groups within the chosen 30 % limit[30].
A very small study (n=12+13=25) has tried to investigate empiric prophylaxis with fluor-quinolone Ofloxacin in patients with abdominal aortic aneurism. However the sample size of this study does not justify any conclusions on this issue[44].

2 TRIAL OBJECTIVES AND ENDPOINTS

2.1 Trial Objectives

2.2 Primary Objectives

To address whether immediate diagnostic and therapeutic initiatives guided by abnormal high and increasing values of Procalcitonin measured daily can reduce the mortality of critically ill patients in the ICU.

2.3 Secondary Objectives

1. To determine mortality of ICU patients at discharge from the ICU, at day 60,90, 120 and 180.

2. To determine differences in prescription of antimicrobial therapy in the two arms.
3. To determine the frequency of patients with complications to infection in the two arms, defined as; sepsis, severe sepsis, septic shock, disseminated intravascular coagulation, multi-organ dysfunction syndrome (MODS), coma (Glasgow Coma Scale), hypotension, respiratory insufficiency (ventilator treatment need), liver insufficiency, acute uremia (three times increase in baseline creatinine).
4. APACHE II score
5. Accumulated PCT increases over time
6. To determine the number of diagnostic image procedures per day after enrolment in the trial in the two arms
7. To determine the number of non-routine microbiological samples taken per day after enrolment in the trial in the two arms
8. To determine the number of surgical procedures per day after enrolment in the trial in the two arms
9. To determine the time to the first change in antimicrobial chemotherapy after admittance to the ICU in the two arms

2.4 Trial Endpoint(s)

Primary:

Mortality at day 28 after admission to the ICU.

Secondary:

1. Mortality while admitted to the ICU, Mortality at day 60, 90 and 180 after admission to the ICU
2. Defined day doses of antimicrobial therapy in each arm
3. Occurrence of sepsis, severe sepsis, septic shock, DIC. Assessment of Glasgow Coma Scale, measurement of Blood Pressure (systolic blood pressure < 90), days with artificial ventilation, Factor 2-7-9 < 0.7, creatinine (increase factor 3 from baseline), MODS.
4. SOFA score daily (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale).

5. $AUC_{Procalcitonin}$ for the Procalcitonin-measuring group and for the control group.
6. Number of diagnostic images after admission to the ICU.
7. Number of non-routine microbiological sample taken after admittance to the ICU.
8. Number of surgical procedures during the trial
9. Time to the first change in antimicrobial chemotherapy after admittance to the ICU

3 INVESTIGATIONAL PLAN

3.1 Trial Design

3.1.1 Intervention

This is a randomised, single-blinded multicentre trial.

Approximately 1000 subjects admitted to an ICU in the participating University hospitals will be included. All patients included will receive the the standard recommended diagnostic and therapeutic procedures mandated at the particular ICU. Additionally, the patients will be randomised for:

1. No PCT guided diagnostics and treatment (i.e. the standard-of-care / **control arm**).

Or

2. Daily PCT measurements and protocol-specified additional diagnostic and/or therapeutic interventions guided by the PCT levels observed. High or increasing PCT levels will mandate such interventions (see section 3.3.1 for details of interventions)(the **PCT intervention arm**)

3.1.2 Randomisation

The randomisation is performed by the PASS study centre and is stratified according to site, age and initial Acute Physiology And Chronic Health Evaluation II (APACHE II) score. For patients randomised to the PCT intervention arm, daily PCT levels are communicated to the team responsible for the clinical management together with a recommendation of what interventions the investigator team is expected to initiate based on the PCT measurement. In

the control arm, blood samples for PCT will be analysed simultaneously with samples from the PCT intervention arm, but results of these PCT analyses will remain blinded for the investigators until the study has been completed. The PCT measurements will be conducted daily as long as the patient is admitted to the ICU, but maximally 28 days from time of enrolment in this study. While patients remain in the hospital, and after discharge from the ICU, samples will be collected for PCT determination but the samples will not be analysed real-time and hence the results will not be used to guide interventions outside the ICU, except if requested by the ICU investigator in conjunction with the discharge of the patient. Patients transferred from one ICU to another ICU, will remain in the trial provided that the receiving ICU also participates in this trial.

3.2 Trial Population

3.2.1 Inclusion Criteria

A subject will be eligible for inclusion in this trial only if all of the following criteria apply:

1 Male or female, aged ≥ 18 years of age.

2 Admitted to the participating intensive care units. Patients should be included within 24 h. If a patient has not been included at this time, this patient cannot be included in the present admittance.

3 Subjects should in the investigator's opinion be likely to be admitted to the ICU for more than 24 h. Subjects should not be likely (<10%) to die or be discharged in this period of time

4 Ability to understand and provide written informed consent to participate in this trial,

or

Ability to understand and provide oral informed consent in presence of at least one impartial witness who should sign and personally date the consent form

or

The subjects legally acceptable representative can understand and provide written informed consent if the subject is not capable of this because of the present mental or physical condition of the subject.

3.2.2 Exclusion Criteria

A subject will **NOT** be eligible for inclusion in this trial if any of the following criteria apply:

4. Subjects with known hyper-bilirubinaemia (>0.4 mg/ ml) or hypertriglyceridaemia (>10 g/l) since this can interfere with measurements. If subjects with unknown status on these points are included and have PCT measurements, the measuring-equipment will detect these conditions.
5. Subjects suffering from a blood disorder, where daily sampling of 7 ml of blood for maximally 28 days (210 ml distributed on 28 days) will be an inconvenience or a potential risk, which could compromise the safety of the subject.

3.3 Treatment During Trial

The aim of the PCT guided treatment is to reduce time to relevant treatment of a serious infection and thereby to reduce the mortality. All subjects will receive the standard-of-care evaluations and therapeutic interventions recommended in the ICU at which the patient is admitted to. Subjects in the PCT measurement group will additionally receive expanded diagnostics and treatment should the PCT levels be found to high and/or increasing (see section 3.3.1 for definitions).

Access to results of PCT measurements of any kind (semi-quantitative or quantitative) at any time in the study period is not allowed for patients randomised to the control arm.

The PASS study group in collaboration with the PASS Steering Committee, will issue guidelines for the composition of the interventions that a high or increasing PCT level would mandate. Some variation between sites is acceptable, whereas all patients within a given ICU should follow that ICU's guidelines. The guidelines will be updated when new information becomes available. In the guidelines, there may be several alternatives indicated for a given situation. The investigator is not mandated to follow the guidelines.

3.3.1 Procalcitonin levels and diagnostic and therapeutic consequenses

The situation mandating additional interventions in the the PCT intervention arm is based on the following criteria:

- PCT levels ≥ 1.00 ng/ml

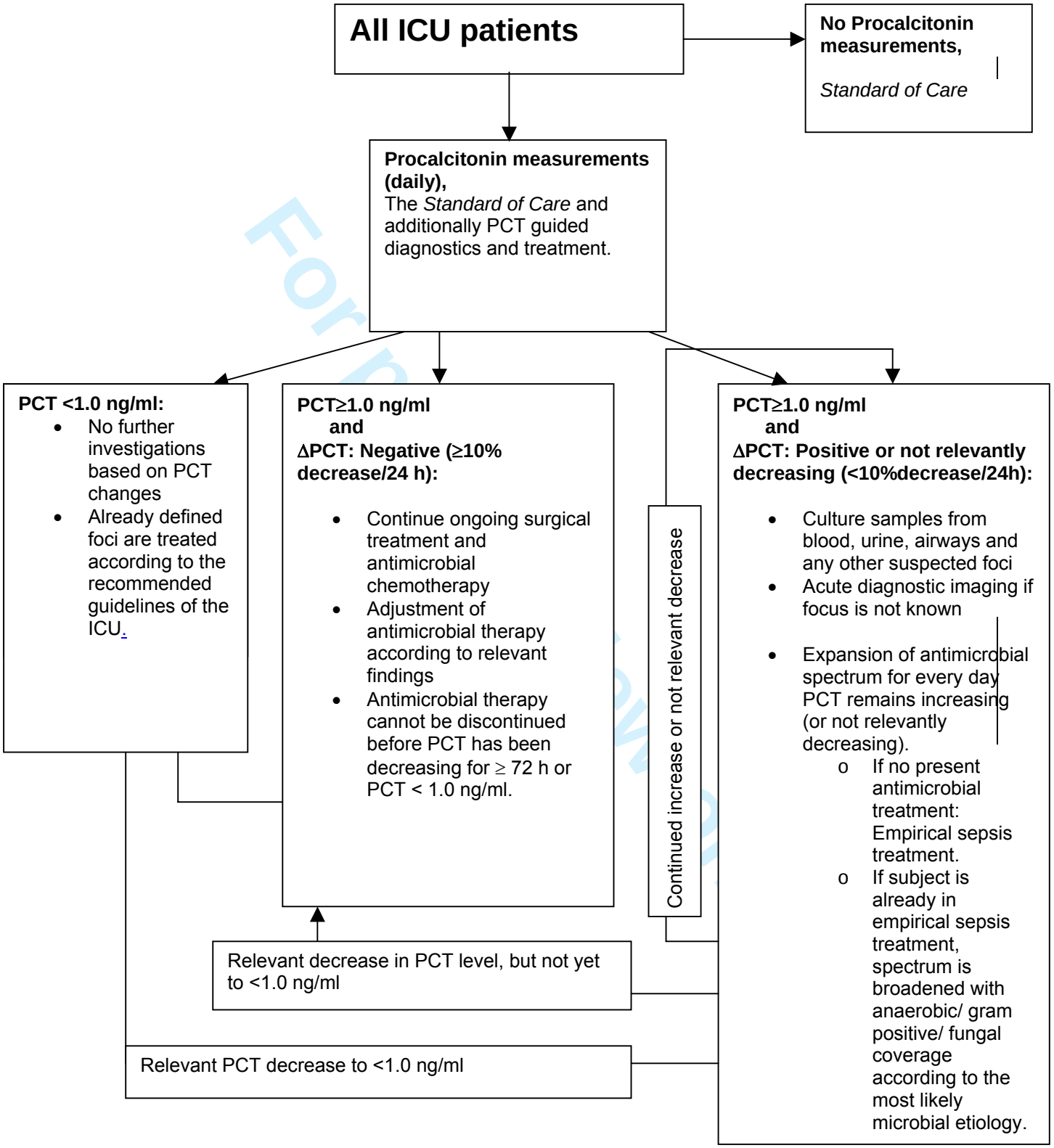
and

- The PCT level increases one day to the next or has an irrelevant decrease of < 10%

The daily assessment of PCT guided interventions will be as follows:

- Subjects with PCT levels ≥ 1.00 ng/ml based on the first determination after enrolment into the study will follow the principles for interventions as detailed below.
- Subjects with PCT levels ≥ 1.00 ng/ml and with a day (n) to day (n+1) PCT increase or a decrease of < 10% (irrelevant decrease) will follow the principles for interventions as detailed below.
    - Microbiology: blood cultures, airway cultures, urine cultures and samples from any other suspected foci.
    - Considerations of whether to perform diagnostic imaging: one or more of the following: Chest X-ray, Ultra-sonic examination of suspected focus, Computerised Tomography of relevant areas, Magnetic Resonance imaging of relevant areas, other imaging techniques.
    - Surgical drainage of possible un-drained foci
    - Antimicrobial therapy expansion. Treatment will be guided by any relevant findings: microbial or diagnostic imaging, or other findings. If focus and micro organism of infection is not clear steps will be:
        1) Empirical sepsis treatment
        2) Empirical sepsis treatment with anaerobic and gram positive coverage
        3) Empirical sepsis treatment with anaerobic and gram positive coverage and/ or fungal treatment
- Subjects with PCT levels < 1.00 ng/ml will continue to receive standard-of-care
- Subjects with PCT levels ≥ 1.00 ng/ml and with a day-to-day PCT **decrease** of ≥ 10% will continue to receive standard-of-care.

Precise guidelines for this (antimicrobial) treatment will be made specifically for every ICU in concordance with the local choices regarding antimicrobial agents. For PCT guided diagnostics and treatment algorithm, see Diagram 1:



- **Antimicrobial treatment is NOT to be discontinued if PCT is increasing and > 1.0 ng/ml**
- **When treatment of infection is relevant, PCT normally decreases in less than 18 h. If PCT is still not decreasing at the next-coming measurement after a therapy shift, a new (expanded) strategy is to be instituted**

3.3.2 Change of PCT-guidance strategy during the trial

3.3.2.1 Randomised PCT-guided interventions

Subjects may **discontinue** the interventions initiated on the basis of PCT measurements only in case the benefit: risk ratio for these interventions is not acceptable to the treating physician. The specific concern will be collected.

3.3.2.2 The non-PCT guided interventions

The recommended interventions based on other information than PCT measurements should always be instituted and continued when relevant from a clinical judgement.

3.3.3 Antimicrobial Drugs and Dosages

All antimicrobial drugs prescribed on basis of an increasing PCT must be prescribed by the investigator or an intensive care physician, who has been sufficiently instructed in all aspects of the trial. The investigator must check for possible drug-drug interactions between any of the drugs prescribed guided by PCT changes and other agents that may be metabolised via the same enzyme systems or organs. To assist the investigator, information on this topic is included in the Manual of Operational Procedures. Also, the product label of each drug prescribed should be reviewed.

General principles that will be followed regarding antimicrobial therapy of sepsis are:

- Antimicrobial agents are prescribed, when possible, according to the resistance pattern of the causative microorganism.
- When the causative microorganism is not known, antimicrobial agents are prescribed according to knowledge of which microorganisms normally and possibly infect the suspected focus.
- When neither the microorganism nor the focus of infection is known, one or more broad spectrum antimicrobial agents are selected. If the effect is not sufficient, the spectrum of the used antimicrobial agents is additionally expanded, often with anaerobic active agents, gram positive active agents and antifungal agents. Conversely, if the effect is sufficient, the spectrum of used antimicrobial agents is narrowed according to knowledge of focus and causative microorganism.
- In empiric sepsis treatment, a combination of a ß-lactam/ Carbapenem + a fluor-quinolone is chosen if not contra indicated in the specific subject. This treatment can be

supplemented with nitroimidazoles, glycopeptides, oxazolidinones and azoles. More specific treatment regimes are initiated and guided by findings regarding the causative microorganism and/or focus of infection.

Dosages of antibiotics are decided according to the recommendations of the specific ICU.

The toxicity management guidelines detailed below refer to all components of the antimicrobial treatment used in the trial.

3.3.3.1 Overdose and Toxicity

Antimicrobial agents may be interrupted because of the development of adverse events (AEs, see section 6.1 for definitions) at the discretion of the investigator and according to the severity of the AE. The dose of all antimicrobial drugs may be reduced, interrupted or reintroduced according to standard practice at the time, and depending on the severity of the AE.

Subjects who require a dose modification should be re-evaluated on a daily basis.

The investigator is responsible for taking appropriate precautions to ensure that the risk of developing toxicity is minimised, that the subject is monitored for the development of toxicity, and if such toxicities do occur, take appropriate action to minimise their effects.

4 MEASUREMENTS AND EVALUATION

4.1 Time and Events Schedule

A flow chart showing the timing of trial procedures (Clinical and Laboratory) is shown in Table 1.

An initial pre-entry (screening) assessment for eligibility will be performed as soon as possible after the patient is admitted to the ICU. The patient should be randomised no later than 24 hours after the time of admission. Evaluations will then be carried out at entry (Day 1), and thereafter daily as long as the patients remains in the ICU. After discharge, the course of disease is collected in less detail and the survival status determined day 28, 60, 90 and 180 after enrolment in the trial.

4.1.1 Pre-entry Evaluations

The site must obtain subject consent in the form of a written informed consent form prior to the initiation of **any** pre-entry procedures as outlined in this protocol. The consent form must be approved by the IEC of each participating site.

The pre-entry evaluation will be conducted the first day of the trial by an investigator in the ICU and will include an evaluation of whether the patient fulfils the requirements for enrolment in this trial (see section 3.2.2 and 3.2.3.

Subjects who fail to meet the entry criteria may not be re-screened for this protocol until 28 days after the failed pre-entry evaluation. Hence, enrolment of such patients will require that the patient is re-admitted to the ICU after at least 7 days outside of the ICU after the time of the first screening.

4.1.2 Baseline (Day 1) Evaluations

The following evaluations should be performed at baseline (Day 1):

Note: For this trial, Baseline (Day 1) is defined as the day on which the subject has his/her first blood sample for PCT measurement. The following data are to be collected on day 1:

- Demography including date of birth, weight, height, and indication for admittance to the ICU
- Infections found in the subject in this hospital admission prior to admittance to the ICU.
- Present infection focus/ etiologic microorganism

- APACHE II score (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale)

- Current medical conditions

- Pre-admittance daily function and health state:

Professional career:	1) Student, 2) Part time work, 3) Full time work, 4) Early retirement, 5) Retired
Health:	1) Congenital handicapped, 2) Acquired handicap, 3) Chronic disabling disease, 4) Chronic non-disabling disease, 5) Healthy
Self-supportance:	1) Lives in nursing home, 2) Lives in a flat connected to a nursing home, 3) Own home with external help ≥ once / day, 4) Own home with external help < once daily, 5) Own home, no help required
Hospital need:	1) ≥ 3 months admitted to a hospital/ last year, 2) 1-3 months admitted to a hospital/ last year 3) 1-30 days admitted/ last year, 4) No admissions, ambulatory visits ≥ 6/ last year, 5) No admissions, ambulatory visits 1-5/ last year, 6) No admissions, No ambulatory visits/ last year

- Adverse events/ other complications to treatment given in this hospital admission (ongoing clinical conditions at Day 1 shall be recorded in the “Adverse Event and Medical Condition Form” of the CRF at this time, regardless of the fact that such conditions may not subsequently be found to fulfil the definitions for an adverse event (see section 6.1))

- Haematology: *haemoglobin, platelet count (WBC count mentioned as part of APACHE II)*

- Clinical chemistry: Albumin, Bilirubin, Factor 2-7-9, Alanin Amino Transferase (ALAT)/ Aspartate Amino Transferase (ASAT), Alcaline Phosphatase, Creatinine, Carbamide, Na^+, K^+, Phosphate, Ca^{2+}, C-reactive protein (some are also mentioned as part of APACHE II).

- Baseline PCT

The daily PCT determination is done real-time at the Department of Clinical Biochemical Department, Hvidovre Hospital, using the EC-approved measuring instruments and reagents. For each subject, the same methodology should be used throughout the trial period. The KRYPTOR® PCT BRAHMS sensitive assay is the accepted standard assay. Other licensed assays may be used instead if judged by the PASS steering committee to have a comparable performance compared to the indicated assay.

4.2 On Trial Evaluations

On trial assessments will be completed at the following time-points unless otherwise specified:

While admitted to the ICU, the following information will be registered unless specified otherwise:

Daily while patient is admitted to the ICU:

- Clinical signs of new (nosocomial) infections
- Microbiological or radiological evidence of new (nosocomial) infection
- Defined Day Doses of antimicrobial chemotherapy
- APACHE II score (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale)
- Occurrence of sepsis, severe sepsis, septic shock, DIC. Assessment of Glasgow Coma Scale, measurement of Blood Pressure (systolic blood pressure < 90), days with artificial ventilation, Factor 2-7-9 < 0.7, creatinine (increase factor 3 from baseline), MODS.
- Adverse events/ other complications to treatment given in the ICU (ongoing clinical conditions at Day 1 shall be recorded in the “Adverse Event and Medical Condition Form” of the CRF at this time, regardless of the fact that such conditions may not subsequently be found to fulfil the definitions for an adverse event (see section 6.1))
- Haematology: haemoglobin, platelet count WBC (WBC count also mentioned as part of APACHE II)
- Clinical chemistry: Albumin, Bilirubin, Factor 2-7-9, Alanin Amino Transferase (ALAT)/ Aspartate Amino Transferase (ASAT), Alcaline Phosphatase, Creatinine, Carbamide, Na^+, K^+, Phosphate, Ca^{2+}, C-reactive protein (some are also mentioned as part of APACHE II).

- Blood sample for PCT determination
- Diagnostic imaging procedures performed
- Non-routine microbiological sample taken
- Surgical procedures performed
- Change in antimicrobial chemotherapy

At the day of discharge from ICU or day of death or later:

- Mortality and time of death, and the cause hereof
- $AUC_{Procalcitonin}$ (at discharge from the ICU) (will remain blinded in the control arm)
- Discharge and post-discharge daily function and health state (obtained on day 30 and 180):

Professional career:	1) Student, 2) Part time work, 3) Full time work, 4) Early retirement, 5) Retired
Health:	1) Congenital handicapped, 2) Acquired handicap, 3) Chronic disabling disease, 4) Chronic non-disabling disease, 5) Healthy
Self-supportance:	1) Lives in nursing home, 2) Lives in a flat connected to a nursing home, 3) Own home with external help ≥ once / day, 4) Own home with external help < once daily, 5) Own home, no help required.
Hospital need:	1) ≥ 3 months admitted to a hospital/ last year, 2) 1-3 months admitted to a hospital/ last year 3) 1-30 days admitted/ last year, 4) No admissions, ambulatory visits ≥ 6/ last year, 5) No admissions, ambulatory visits 1-5/ last year, 6) No admissions, No ambulatory visits/ last year

After discharge from ICU while patient is still admitted to hospital

- Clinical signs of new (nosocomial) infections

- Microbiological or radiological evidence of new (nosocomial) infection
- Defined Day Doses of antimicrobial chemotherapy
- Current medical conditions (including acute organ failures)
- Diagnostic imaging procedures performed
- Surgical procedures performed
- Blood sample for PCT determination – done daily

4.3 Trial drugs

Drugs prescribed on basis of PCT levels and changes belong to following categories: Antibacterial chemotherapeutics and Antifungal chemotherapeutics. Drugs from these categories will also be prescribed for the control group (and in patients not included in the trial), when indicated from other findings than level/change of PCT. An exhaustive list of drugs, used in the participating ICU´s (and thereby also in the trial subjects and controls) is given in appendix

4.3.1 Dosing Details

The following details on dosing of all prescribed antimicrobials during the study period must be recorded in the “Medication form” in the CRF.

- Date of initial therapy
- Dose at each dosing change, together with reason for change
- Date of last dose of each agent
- Reason for discontinuation
- Date of resumption of therapy

4.3.2 Collection of Blood Samples for Daily Analysis

Plasma from the PCT group and the control group will be collected early each morning (01.00 a.m.-06.00 a.m.) and will be transported to the Department of Clinical Microbiology Hvidovre Hospital, DK-2650 Hvidovre (or other laboratories, that can provide a PCT analysis real-time and with an analysing method which is approved by the PASS coordinating centre) and analysed immediately hereafter. The results from this analysis will be communicated via a

webbased cryptized licensed answering system every day to the Intensive Care Units for patients randomised to the PCT intervention arm or concealed for patients randomised to the control arm. Remaining material for the blood samples will hereafter be frozen for later analysis of other biochemical, biological and genetic markers (-80°C). Once the trial has been completed, the coupling of these samples to person-identifiers will be broken, and hence subsequent analyses done without any possibility to connect the results to individual persons involved in the trial. For detailed instructions regarding the collection, labelling, processing and transport of samples, see the Manual of Operational Procedures.

It is the responsibility of the investigator (to be assisted by the courier service and PASS coordinating office) to ensure that all trial samples for transport are appropriately handled, packed and transported.

4.3.3 Genetic markers (PASS-sub-study)

The PASS-sub-study has three aims: 1. quality assessment of the procalcitonin analyzes used in the PASS-Study, 2. to investigate the relation between levels of procalcitonin and other biomarkers and 3. to investigate if genetic markers can be used to gain an early knowledge of the course of critical illness.

To investigate this, we will use the remaining material from the blood samples collected for the PASS-Study. Blood plasma and DNA material will be frozen at minus 80 degrees Celcius. The PASS-Sub-study, therefore, will not mean any inconvenience for the study subjects and no additional blood sampling. This material will be kept in anonymous form for 5 years after the closure of the PASS-Study. Known hereditary diseases will not be examined.

Regarding 1.: In a randomly assigned set of blood samples, and additionally in samples that have shown extreme PCT values a double determination will be performed to assess the inter-assay variability.

Regarding 2.: Other biomarkers as interleukin-6 and soluble TNF-α receptor have been, and are still under assessment as predictive markers at sepsis and in other infectious diseases. In plasma, these and other markers will be analyzed after the closure of the PASS-Study to assess the value of these markers compared to PCT, also as prognostic markers.

Regarding 3.: Genetic polymorphisms (e.g. mannan-binding lectins, interleukins, complement, immunglobulin receptor, Toll-like receptor 1-9, and Factor V Leiden) are related to the prognosis at sepsis and can, to some degree, identify patient groups with a high risk of a fatal course of

the disease. An increasing number of international studies have during the latest years investigated the relation between the genetic disposition of patients and the course of infectious diseases, but often, these studies have been small and without sufficient statistical power to conclude on these issues.
The statistical power in investigating the relation between genetic polymorphisms and mortality in sepsis depends on the frequency of a certain allele, the mortality in the study population and the size of the population.

Directly applied on the study population of the PASS-Study with 1000 cases of sepsis (mortality ~25%) it will result in a 80 % statistical power to show a 2-fold increase in mortality for an allele that is found in 3% of the population. For alleles that are more frequent, we will be able to show less than a 2-fold increase in mortality. As an example of this, the homozygote forms of TNF-α, IL-1β, and PAI-1 have a frequency of 5, 7, and 14%, respectively. Heterozygote forms of TLR4 and factor V Leiden have a frequency of 9 and 7%.

5 DATA ANALYSIS METHODS

5.1 Sample Size Determination

The trial will randomise (1:1) 1,000 subjects into two treatment arms:

1: Control arm

2: The PCT guided intervention arm

With a sample size of 500 per group and an assumed mortality rate of 25% in the control group and 17.5 % in the PCT group there will be 80% probability that a negative result (Confirming the Null Hypothesis) is true. At the same time there will be < 5% probability of falsely declaring the alternative hypothesis correct. [Power 80%, stringency 5%]. Sample Size calculations via Dept. of Statistics, UCLA, California, USA.

5.2 General Considerations

5.2.1 Analysis Populations

The primary population for analyses of the efficacy and safety data will be the intention to treat population, including all randomised subjects who have at least one blood sample made for PCT measurements.

Response to PCT guided diagnostic and therapeutic interventions will also be investigated descriptively by summary statistics for various sub-groups, e.g. gender, other demographic variables, Baseline APACHE II score, and pre-admittance health assessment.

5.2.2 Interim Analysis

Safety and efficacy data will be reviewed when 250, 500 and 750 subjects have completed the trial period (until discharge from the hospital or death, maximally 28 days), or at least every 6 th month, and assessments will be made by an independent Data and Safety Monitoring Board (DSMB). A cut-off date will be specified at this point and all treatment failure and adverse event data before this date will be used.

The Peto method of repeated significance testing will be used to test for treatment difference and a p-value of 0.001 will be used as the significance level at the interim analysis, giving a significance level of 0.05 for the final analysis once all patients have completed the trial.

Stopping the trial will not be based purely on a statistical decision but also on the recommendation of the DSMB.

5.2.3 Other Issues

All subjects will remain in the trial and be followed-up until day 180.

5.3 Efficacy

5.3.1 Primary Efficacy Endpoint

The primary efficacy analysis will be the comparison of the two treatment groups with respect to the incidence of mortality within 28 days after enrolment in the trial. Mortality is defined as all-cause mortality. Subjects not followed for the entire duration of the trial (i.e. lost to follow-up) will be counted as survivors. Very few patients will be lost to follow up for the primary endpoint, because of the Danish Central Person Register (CPR), where all deaths in Denmark are registered. Only subjects who permanently move their address to another country within 30 days after ICU admission can be lost to follow-up. The stratified log-rank test and Kaplan Meier estimates will be used.

5.3.2 Secondary Efficacy Endpoint(s)

5.3.2.1 Other mortality assessments

The proportion of subjects, who survive to different points of time (at discharge, after 60, 90 and 180 days, counting after ICU admission). The log rank test and Kaplan-Meier estimates will be used. Differences in proportions of survivors will be assessed using the Mantel-Haenzel Chi Square test and Wilcoxon test. Subjects with missing mortality data will be classified as survivors.

5.3.2.2 Other parameters than mortality

- Defined day doses of antimicrobial therapy in each arm
- Occurrence of sepsis, severe sepsis, septic shock, DIC. Assessment of Glasgow Coma Scale, measurement of Blood Pressure (systolic blood pressure < 90), days with artificial ventilation, Factor 2-7-9 < 0.7, creatinine (increase factor 3 from baseline), MODS.
- SOFA score daily (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale).
- $AUC_{Procalcitonin}$ for the Procalcitonin-measuring group and for the control group.
- Number of diagnostic images after admission to the ICU.
- Number of non-routine microbiological sample taken after admittance to the ICU.
- Number of surgical procedures during the trial
- Time to the first change in antimicrobial chemotherapy after admittance to the ICU
- Occurrence of new clinically, microbiologically or radiologically diagnosed infections while admitted to the ICU
- Discharge and post-discharge daily function and health state

For endpoints that have normally distributed numbers, t-test will be used in assessment of statistical significance. If not normally distributed, Mantel-Haenzel Chi Square test and the Wilcoxon test, will be used.

Exploratory analysis of adjustments for possible confounders present at baseline for the analysis presented above will be performed using Cox proportional hazards and Logistic regression modelling (as appropriate).

5.3.3 Combined evaluation of mortality / occurrence of serious bacterial infection while admitted to the ICU

The proportion of patients who die during the trial period or who experience occurrence of a serious bacterial infection (sepsis, severe sepsis, septic shock, Disseminated Intravascular Coagulation (DIC) or Multi Organ Dysfunction Syndrome (MODS) (which ever came first) as a function of time since trial initiation. In this analysis, patients discontinuing the randomised treatment for other reasons before having failed in this analysis will be censored from the time of discontinuation.

5.4 Safety

Adverse events will be tabulated by treatment group, maximum intensity, attributability to various antimicrobial agents and by seriousness. Treatment related adverse events that lead the subject to prematurely discontinue one or more of the originally prescribed antimicrobial agents will also be summarised.

Clinical chemistry and haematology results will be presented by summary statistics and quartile plots of measured results. Change from baseline for these results will also be presented. Baseline is defined as the laboratory data collected at Day 1 (before the first blood sample for PCT analysis). Subjects must have both a baseline and an “on treatment” measurement to be included in the change from baseline analysis.

Treatment emergent toxicity grades will be presented for each graded laboratory parameter by treatment group. A graded toxicity is considered treatment emergent if it develops or increases in intensity, post Day 1. Treatments will include established and approved antimicrobial treatments, which are already used daily in the participating ICU´s.

Concurrent medications and blood products will be summarised by randomised treatment group.

6 ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

As mentioned other places in this protocol, the direct inconvenience for subjects in this study is sampling of 7 ml of whole blood daily in the same session as the routine blood samples are made, every morning. Therefore it is reasonable to expect that AE´s and SAE´s as a direct consequence of this blood sampling will not occur. Indirect AE´s as a consequence of potential overly treatment are likewise not likely to occur according to the available literature on the issue, especially because the most striking result of the previously published RCT´s is a reduction of antibiotic exposure in the PCT-guided group.
All interventions, that are performed in this study are well-known, thoroughly tested and accepted treatments, so it does not seem reasonable to apply the same procedures for this study regarding AE´s as e.g. a study where a new drug is to be assessed for safety (or effect)

Investigators will, however, have the opportunity to report events, that they fing unexpected in the Case Report Form. In this part of the CRF, it is possible to classify unexpected events in groups of "relatedness" to the antimicrobial treatment as "no relation", "unlikely relation", "possibly related", "probably related" or "definitely related.

Serious unexpected events or unexpected events

Serious inexpected events and unexpected events, that can be related to the antimicrobial treatment will in both treatment groups be reported to the Danish Medicines Agency "Lægemiddelstyrelsen" according to the Danish legislation on this point

The primary and the secondary endpoints that are registered daily in the case report form are all *adverse events or serious adverse events, i.e. death, complications to sepsis, increased antibiotic exposition and prolonged hospital stay. These are registered routinely and daily in the part of the CRF dealing with effects of the treatments. All patients are at inclusion in the study threatened by potentially lethal illnesses.*

7 TRIAL ADMINISTRATION

7.1 Data Collection

Case Report Forms (CRF) will be provided for each subject by the PASS coordinating centre. All data on the CRFs must be entered legibly in black ink or typed, in Danish or English. Amendments and errors on the CRFs should not be erased, covered with correction fluid or completely crossed-out; rather, a single line should be drawn through the error and the correction initialled and dated by the investigator, authorised colleague or co-worker. An explanatory note for the change should also be written on the CRF. Any requested information which is not obtained or unanswerable should be identified by entering 'ND' (not done). An explanation must be documented for any missing data. CRFs must be completed regularly and should never bear the participant's name. Participants will be identified by initials, date of birth and subject trial number only.

The investigator (or a person appointed by the investigator) must sign and date a declaration on the CRF attesting to his/her responsibility for the quality of all data recorded and that the data represents a complete and accurate record of each subject's participation in the trial.

Details and procedures for the completion of the CRFs are specified in the Manual of Operational Procedures.

All trial CRFs will be plain paper copies – the original being the investigators copy. After completion of each page of the CRF, the investigator will send it by fax to the PASS coordinating centre. Pages will be reviewed and clarified in accordance with the protocol specific Review and Validation Manual. The data will be double entered (punched and verified) by separate data entry specialists to produce data files.

Identical validation checks will be performed on each database. Data failing any check will be flagged for output on a Data Clarification Report (DCR) and sent to the relevant investigator for resolution. In such cases the investigator is requested to sign and date any explanation or correction. On return, the database will be updated appropriately and the original DCR stored with the original CRF.

The database(s) will be subject to agreed Quality Control (QC) checks before authorisation. The data will be subsequently analysed according to the methods outlined in Section 5.

7.2 Regulatory and Ethical Considerations

7.2.1 Regulatory Authority Approval

The co-ordinator (in collaboration with the PASS coordinating centre) will obtain approval from the appropriate regulatory agency prior to initiating the trial at a site.

This trial will be conducted in accordance with ICH-GCP and all applicable regulations, including, where applicable, the Declaration of Helsinki, June 1964, as modified by 52nd WMA General Assembly, Edinburgh, Scotland, October 2000 (see Appendix 1).

7.2.2 Ethics Approval

It is the investigator's responsibility to ensure that this protocol is reviewed and approved by the appropriate local Independent Ethics Committee (IEC). The IEC must also review and approve the site's informed consent form (ICF) and any other written information provided to the subject prior to any enrolment of subjects, and any advertisement that will be used for subject recruitment. The co-ordinator and/or the investigator must forward to the PASS coordinating centre copies of the IEC approval and the approved informed consent materials, which must be received by the PASS coordinating centre prior to the start of the trial.

If, during the trial, it is necessary to amend either the protocol or the informed consent form, the co-ordinator and/or investigator will be responsible for ensuring the IEC reviews and approves these amended documents. IEC approval of the amended ICF must be obtained before new subjects consent to take part in the trial using this version of the form. Copies of the IEC approval of the amended ICF and the approved amended ICF must be forwarded to the PASS coordinating centre as soon as available.

7.2.3 Subject Informed Consent

The investigator or his/her designee will inform the subject of all aspects pertaining to the subject's participation in the trial.

The process for obtaining subject informed consent will be in accordance with all applicable regulatory requirements. The investigator or his/her designee and the subject/ witness of an oral informed consent/ subjects legally acceptable representative must both sign and date the ICF before the subject can participate in the trial. Following types of informed consent can be accepted because of the nature of the ICU setting and the physical and/ or mental state of the subjects.

1)Ability to understand and provide written informed consent to participate in this trial,

or

2)Ability to understand and provide oral informed consent in presence of at least one impartial witness who should sign and personally date the consent form

or

3)The subjects legally acceptable representative can understand and provide written informed consent if the subject is not capable of this because of the present mental or physical condition of the subject.

The subject will receive a copy of the signed and dated form and the original will be retained in the site trial records. The decision regarding subject participation in the trial, that is made by the subject, is entirely voluntary. The investigator or his/her designee must emphasize to the subject that consent regarding trial participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

If the ICF is amended during the trial, the investigator must follow all applicable regulatory requirements pertaining to approval of the amended ICF by the IEC and use of the amended form (including for ongoing subjects).

7.3 Trial Monitoring

In accordance with applicable regulations, good clinical practice (GCP), monitors will periodically contact the site, including conducting on-site visits. The extent, nature and frequency of on-site visits will be based on enrolment rate, the quality of the documents provided by the site, consistency of follow-up of the patients according to this protocol.

During these contacts, the monitor will:

- check and assess the progress of the trial

- review trial data collected
- conduct Source Document Verification
- identify any issues and address their resolution

This will be done in order to verify that the:

- data are authentic, accurate, and complete
- safety and rights of subjects are being protected
- trial is conducted in accordance with the currently approved protocol (and any amendments), GCP, and all applicable regulatory requirements

The investigator agrees to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and any relevant issues.

In addition to contacts during the trial, the monitor will also contact the site prior to the start of the trial to discuss the protocol and data collection procedures with site personnel.

At trial closure, monitors will also conduct all activities as indicated in Section 7.5, Trial and Site Closure.

7.4 Quality Assurance

At its discretion, the PASS coordinating centre may conduct a quality assurance audit of this trial. If such an audit occurs, the investigator agrees to allow the auditor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor to discuss findings and any relevant issues. A guideline for audit is available at the PASS coordinating centre.

In addition, regulatory agencies may conduct a regulatory inspection of this trial. If such an inspection occurs, the investigator agrees to allow the inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the inspector to discuss findings and any relevant issues.

7.5 Trial and Site Closure

Upon completion of the trial, the following activities, when applicable, must be conducted by the monitor in conjunction with the investigator, as appropriate:

- return of all trial data to the PASS coordinating centre

- data clarifications and/or resolutions
- review of site trial records for completeness
- shipment of stored samples to assay laboratory

In addition, the steering committee reserves the right to temporarily suspend or prematurely discontinue this trial either at a single site or at all sites at any time and for any reason. If such action is taken, selected members of the PASS steering committee and/or the PASS coordinating centre will discuss this with the Investigator (including the reasons for taking such action) at that time. The PASS coordinating centre will promptly inform all other investigators conducting the trial if the trial is suspended or terminated for safety reasons. The investigators will inform their local/regional/national regulatory authorities (as appropriate) of the suspension or termination of the trial and the reason(s) for the action. If required by applicable regulations, the investigator must inform the IEC promptly and provide the reason for the suspension or termination.

If the trial is prematurely discontinued, all trial data must be returned to the PASS coordinating centre.

7.6 Records Retention

In accordance with applicable regulatory requirements, following closure of the trial, the investigator will maintain a copy of all site trial records in a safe and secure location. The PASS coordinating centre will inform the investigator of the time period for retaining these records in order to comply with applicable regulatory requirements.

7.7 Information Disclosure and Inventions

7.7.1 Confidentiality

The investigator and other trial site personnel will keep confidential any information provided by the co-ordinating centre (including this protocol) related to this trial and all data and records generated in the course of conducting the trial, and will not use the information, data, or records for any purpose other than conducting the trial. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the investigator or trial site personnel; (2) information which it is necessary to disclose in confidence to an IEC solely for the evaluation of the trial; or (3) information which it is necessary to disclose in order to provide appropriate medical care to a trial subject.

7.7.2 Publication

The findings from this trial is intended to be published in peer-reviewed journals. The steering committee decides whether abstracts are to be submitted to conferences, and how the results are distributed if more than one manuscript is to be drafted.

Authorship: The trial group as a whole will appear in an appendix in all published manuscripts. Co-authors are selected after a fair evaluation of primarily number of patients entered in to the trial and the level of involvement in the drafting of the manuscript. Providing that several manuscripts are to be drafted, a fair rotation among the participating clinical sites of co-authorship slots will be done taking in to consideration the number of patients enrolled.

7.8 Indemnification and Compensation for Injury

The insurance that covers liability in relation to patient care in Denmark, *Patientforsikringen* will cover all liability aspects of the conduct of this trial[45-46].

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Table 1: Clinical and laboratory Evaluations

Evaluation	Day (screening & baseline)		Day (counting after admission to ICU) (follow-up)				
	1	Day=Dis-charge/ death	28	30	60	90	180
Informed Consent	X						
Entry Criteria	X						
Demography	X						
APACHE II	X	X					
Infections during this hospital admission	X						
Current medical conditions	X	X					
State of daily function and health	X			X			X
Mortality		(X)	X		X	X	X
Baseline PCT	X						
$AUC_{procalcitonin}$		X					
Concurrent Medications[a]	X	X		X	X	X	X
Haematology	X	X					
Clinical chemistry	X	X					
Adverse events	X[a]	X					
Serious Adverse Events	X[a]	X		X	X	X	X

a Adverse events and serious adverse events are registered daily

9. APPENDICES

Appendix 1

Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.
9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.
19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
20. The subjects must be volunteers and informed participants in the research project.
21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.
23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain

informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Appendix 2: Abbreviations

AE	**Adverse Event (AE)**
ALAT	**Alanine Aminotransferase (SGOT)**
APACHE II	**Acute Physiology And Chronic Health Evaluation II**
ASAT	**Aspartate Aminotransferase (SGPT)**
CDC	**Centers for Disease Control**
CRF	**Case Report Form**
DDD	**Defined Day Doses**
DIC	**Disseminated Intravascular Coagulation**
DSMB	**Data Safety Monitoring Board**
ICU	**Intensive Care Unit**
IEC	**Independent Ethics Committee**
IL-6	**Interleukin 6**
MODS	**Multi Organ Dysfunction Syndrome**
PASS	**Procalcitonin and Surivival Study**
PCT	**Procalcitonin**
SAE	**Serious Adverse Event**
TNFα	**Tumor Necrosis Factor α**
WBC	**White Blood cell Count**

Appendix 3: Table of conversion factors for laboratory units

TEST	CONVENTIONAL		SI	
	Unit	Factor	Unit	Factor
Haemoglobin	g/dl	0,6206	mmol/l	1,61
Platelets	Thou/mm^3	0,001	[a]x10^9/l	1000
Hyponatraemia (↓ Sodium)	mEq/l	1,0	mmol/l	1,0
Hypernatraemia (↑ Sodium)	mEq/l	1,0	mmol/l	1,0
Hypokalaemia (↓ Potassium)	mEq/l	1,0	mmol/l	1,0
Hyperkalaemia (↑ Potassium)	mEq/l	1,0	mmol/l	1,0
Hypoglycaemia (↓ Glucose)	mg/dl	0,0555	mmol/l	18,0
Hyperglycaemia (↑ Glucose)	mg/dl	0,0555	mmol/l	18,0
Hypocalcaemia (↓ Calcium)	mg/dl	0,2495	mmol/l	4,0
Hypercalcaemia (↑ Calcium)	mg/dl	0,2495	mmol/l	4,0

[a] No SI unit

For example: Haemoglobin 9,5 g/dl - multiply by factor 0,6206 → 5,9 mmol/l

Appendix 4: Table with the used antibacterial and antifungal drugs used in the 6 participating Intensive Care Units.

Generic name	Comercial name (s)
Benzyl-Penicillin	Penicillin"Leo", Penicillin"Rosco" Benzyl-Penicillin"Panpharma"
Phenoxymethyl-Penicillin	Calcipen ®, Pancillin ®, Primcillin ®, Rocilin ®, Vepicombin ®"DAK"
Dicloxacillin	Dicillin ®, Diclocil ®
Flucloxacillin	Heracillin
Amoxicillin	Amoxicillin"NM", Flemoxin Solutab ®, Imacillin ®, Imadrax ®,
Amoxicillin+Clavulanic Acid	Bioclavid, Bioclavid Forte, Spektramox ®
Ampicillin	Ampicillin"Vepidan", Doktacillin, Pentrexyl ®
Piperacillin	Ivacin ®, Pipril
Piperacillin+Tazobactam	Tazocin ®
Pivampicillin	Pondocillin ®
Pivmecillinam/ Mecillinam	Selexid ®
Cefalexin	Keflex ®
Cefalotin	Keflin ®
Cefepim	Maxipime ®
Cefotaxim	Claforan ®
Ceftazidim	Fortum ®
Ceftriaxon	Rocephalin ®
Cefuroxim	Zinacef, Cefuroxim Stragen, Zinnat ®
Aztreonam	Azactam ®
Meropenem	Meronem ®
Imipenem+cilastatin	Tienam ®
Azithromycin	Zitromax ®
Clarithromycin	Klacid ®, Klacid ® Uno, Klaricid, Zeclar
Erythromycin	Abboticin ®, Abboticin ® Novum, Erycin ®, Escumycin, Hexabotin ®
Roxithromycin	Surlid ®, Forimycin ®, Roximstad, Roxithromycin"Copyfarm", Roxithromycin"UNP"
Doxycyclin	Vibradox ®
Lymecyclin	Tetralysal ®
Oxytetracyclin	Oxytetral ®
Tetracyclin	Tetracyclin"AL", Tetracyclin"DAK", Tetracyclin"SAD"

Gentamicin	**Garamycin ®, Gentacoll ®, Hexamycin, Septopal, Septopal Mini**
Netilmicin	**Netilyn**
Tobramycin	**Nebcina ®, Tobi ®**
Moxifloxacin	**Avelox**
Ciprofloxacin	**Ciproxin ®, Cifin, Ciprofloxacin"1A Farma", Ciprofloxacin"2K Pharma", Ciprofloxacin"Alpharma", Ciprofloxacin"Biochemie", Ciprofloxacin"Gea", Ciprofloxacin"Ratiopharm", Sancipro, Sibunar ®**
Ofloxacin	**Tarivid ®**
Norfloxacin	**Zoroxin ®**
Methenamin	**Haiprex**
Nitrofurantoin	**Nitrofurantoin"DAK", Nitrofurantoin"SAD"**
Sulfamethizol	**Lucosil ®, Sulfametizol"SAD", Sulfametizol"Ophtha"**
Trimethoprim	**Monotrim ®, Trimethoprim"1A Farma", Trimopan**
Sulfamethoxazol+Trimethoprim	**Sulfamethoxazol+Trimethoprim"SAD", Sulfotrim ®**
Clindamycin	**Dalacin ®**
Colistin	**Colimycin**
Teicoplanin	**Targocid ®**
Vancomycin	**Vancocin, Vancomycin"Abbott", Vancomycin"Alpharma"**
Fusidinsyre	**Fucidin ®**
Linezolid	**Zyvoxid ®**
Metronidazol	**Flagyl ®, Metronidazol"Alpharma", Metronidazol"DAK", Metronidazol"SAD"**
Amphotericin B	**Abelcet, AmBisome, Fungizone**
Caspofungin	**Cancidas ®**
Fluconazol	**Conasol, Diflucan ®, Fluconazol"Alpharma", Fluconazol"Copyfarm", Fluconazol"Nycomed", Fluconazol"Ratiopharm", Fluconazol"Stada", Fungal ®, Fungustatin**
Flucytosin	**Ancotil**
Ketoconazol	**Nizoral ®**
Voriconazol	**Vfend**
Ethambutol	**Myambutol ®**
Isoniacid	**Isoniacid"OBA"**
Pyrazinamid	**Pyrazinamid"Medic", Pyrazinamid"SAD"**
Rifabutin	**Rifabutin"Pharmacia"**
Rifampicin	**Rimactan ®**

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts[21 31])	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	1,5,15
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6 + fig. 2 + Diagram D1
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	7-8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5

Section/Topic	Item No	Checklist item	Reported on page No
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6
	11b	If relevant, description of the similarity of interventions	6
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	6-7
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	6-7
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1 (CONSORT diagram
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1 (CONSORT diagram
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	8
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	8-9, table 3 +table 4
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect	

Section/Topic	Item No	Checklist item	Reported on page No
		size and its precision (such as 95% confidence interval)	9-10 + table 2, 3, 4 + fig. 3+4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Abstract + p.
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Table 3, fig. 3+4, p 10.
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms[28])	Table 3+4, p. 10-11, fig. 3+4
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	10-14
Other information			
Registration	23	Registration number and name of trial registry	4-5
Protocol	24	Where the full trial protocol can be accessed, if available	5
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration[13] for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials,[11] non-inferiority and equivalence trials,[12] non-pharmacological treatments,[32] herbal interventions,[33] and pragmatic trials.[34] Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Kidney failure related to broad-spectrum antibiotics in critically ill patients: secondary end point results from a 1200 patient randomized trial

Corresponding author Jens-Ulrik Jensen, Copenhagen HIV Programme, The Panum Institute, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen N, juj@cphiv.dk

Jens Ulrik Jensen *medical doctor*[1,2], Lars Hein *anaesthetist*[3,4], Bettina Lundgren *centre director, hospital diagnostic centre* [2,5], Morten Heiberg Bestle *anaesthetist* [4], Thomas Mohr *anaesthetist*[6], Mads Holmen Andersen *anaesthetist*[7], Klaus Julius Thornberg *anaesthetist*[6], Jesper Løken *anaesthetist*[8], Morten Steensen *anaesthetist*[8], Zoe Fox *biostatistician*[1,9], Hamid Tousi *anaesthetist*[10], Peter Søe-Jensen *anaesthetist*[10,] Anne Øberg Lauritsen *anaesthetist*[3], Ditte Gry Strange *anaesthetist*[3], Nanna Reiter *anaesthetist* [11], Katrin Thormar *anaesthetist*[6], Paul Christian Fjeldborg *anaesthetist* [7], Kim Michael Larsen *anaesthetist*[12], Niels-Erik Drenck *anaesthetist*[11] Maria Egede Johansen *junior research associate*[1], Lene Ryom *junior research executive*[1], Christian Østergaard *senior research executive*[2,13], Jesper Kjær *database manager*,[1] Jesper Grarup *administrative leader* [1], Jens D. Lundgren *professor of infectious diseases*,[1,14] of the The Procalcitonin And Survival Study (PASS) Group*.

[1]Copenhagen HIV Programme at the University of Copenhagen; [2]Department of Clinical Microbiology at Copenhagen University Hospital Hvidovre; [3]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Glostrup; [4]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Hillerød; [5]Diagnostic Centre at Copenhagen University Hospital Rigshospitalet; [6]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Gentofte; [7]Department of Anesthesia and Intensive Care at Aarhus University Hospital in Skejby; [8]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Hvidovre; [9]Royal Free Hospital School of Medicine in London; [10]Department of

Anesthesia and Intensive Care at Copenhagen University Hospital Herlev; [11]Department of Anesthesia and Intensive Care at Copenhagen University Hospital in Roskilde; [12]Department of Anesthesia and Intensive Care at Aarhus University Hospital in Aarhus; [13]Department of Clinical Microbiology at Copenhagen University Hospital Herlev; [14]Department of Infectious Diseases at Copenhagen University Hospital Rigshospitalet. All except[9] are from Denmark. [9] is from England.

*Participating investigators are listed in the appendix.

Running Title: Broad-Spectrum Antibiotics and Renal Failure in Critically Ill Patients

Keywords: Antibiotics – Renal Failure – Sepsis – Intensive Care

Abstract

Objectives: To explore ~~determine~~ whether a strategy of more intensive antibiotic therapy with antibiotics not normally considered to be nephrotoxic leads to adverse renal outcomes in intensive care patients.

Design: Secondary analysis from a randomized antibiotic strategy trial (the *PASS study*). The randomized arms were conserved from the primary trial for the main analysis.

Setting: Nine mixed surgical/medical intensive care units across Denmark.

Participants: 1200 adult intensive care patients, 18 years or older, who were expected to stay more than 24 hours. Exclusion criteria were known ~~extreme~~ bilirubin >40 mg/dL or triglycerides >1000 mg/dL, patients at an increased risk from blood sampling, pregnant or breast feeding and persons ~~held by force (~~psychiatric patients~~)~~.

Interventions: Patients were randomized either to guideline-based therapy ('standard-exposure'-arm), or to guideline-based therapy supplemented with antibiotic escalation whenever procalcitonin increased ('high-exposure'-arm), according to daily measurements of this biomarker.

Main outcome measures: The primary endpoint was estimated GFR<60 ml/min/1.73 m^2. Secondary endpoints were a) delta eGFR after starting/stopping a drug, b) RIFLE criterion *Risk* "R".~~Renal failure, as defined by 1) RIFLE criteria, 2) estimated Glomerular Filtration Rate (eGFR) increase after administration of a certain drug, 3) eGFR) <60 ml/min/1.73 m^2 ('ever' or 'total time') until day 28.~~ Analysis was by intention to treat.

Results: 28-day mortality was 31.8% and comparable (Jensen et al, CCM 2011). A total of 3672/7634 (48.1%) study days during follow-up in the ~~"~~'high-exposure'~~"~~ vs. 3016/6949 (43.4%) in the 'standard-exposure'-arm were spent with eGFR <60 ml/min/1.73m^2, p<0.001. In a multiple effects model, piperacillin/tazobactam was identified as causing the lowest rate of renal recovery of all antibiotics: 1.0 ml/min/1.73 m^2 per 24h while exposed to this drug [95% CI: 0.7 – 1.3 ml/min/1.73 m^2/24h] vs. meropenem: 2.9 ml/min/1.73 m^2/24h [2.5 – 3.3 ml/min/1.73 m^2/24h]);

after discontinuing piperacillin/tazobactam, the renal recovery rate increased: 2.7 ml/min/1.73 m^2 /24h [2.3 – 3.1 ml/min/1.73 m^2 /24h]). eGFR<60 ml/min/1.73m^2 in the two groups at entry and at last day of follow-up was 57% vs. 55% and 41% vs. 39%, resp.

Conclusions: Piperacillin/tazobactam was identified as a cause of delayed renal recovery in critically ill patients. This nephrotoxicity was not observed when using other beta-lactam antibiotics. It remains unclear, whether such a nephrotoxic effect is also present in non-critically ill patients.

Trial registration ClinicalTrials.gov identifier NCT00271752.

Introduction

Frequent complications to sepsis are organ failure, especially respiratory failure and renal failure [1-3]. Critically ill patients are more vulnerable to organ-related drug toxicities than less severely ill patients[4]. Randomized trials assessing safety of broad-spectrum antibiotics in intensive care settings are generally scarce, do not have sufficient statistical power for assessing organ failure endpoints, and do often not include defined kidney organ failure endpoints[5-7]. Data on renal failure endpoints are also sparse in the published trials from other patient populations, and since the absolute risk of renal failure is low for these patients, analyses may likely have been underpowered[8-12].

To our knowledge, randomized trials comparing 'high exposure' vs. 'standard exposure to antibiotics' and specifically addressing whether these interventions affect the occurrence and duration of kidney failure have not been done before in intensive care settings.

In this secondary analysis from a randomized trial, the PASS study[13], we aimed to explore~~investigate~~ whether a strategy of more intensive antibiotic therapy leads to adverse renal outcomes within 28 days after recruitment.

In our study population (and often in severely infected ICU patients), a bacterial hit has resulted in acute onset renal failure, and this bacterial hit (and related organ failure) is often the reason for ICU

admittance. In such situations, with the correct treatment of the underlying infection, we expect renal function to recover. "Lack of recovery" is a non-desirable situation, which may be very serious for the patient. We wanted to explore this, and realizing, RIFLE/AKIN could not capture this, we have used eGFR<60 ml/min/1.73 m^2 as the primary endpoint and examined this from different angles (eGFR<60 ml/min/1.73 m^2 at day 7, days with ml/min/1.73 m^2 . The multiple effects model was built to capture actual estimates of renal function improvement using different antibiotics and adjusting for other known or suspected causes of renal dysfunction.

Secondly, if renal failure was observed from the 'high exposure' approach, to identify one or several of the antibiotics used in this trial as the cause of such a renal failure.

Methods

Trial design and participants

PASS is a multicentre randomized controlled trial in Denmark 2006-9 in 1200 adult critically ill patients, expected to stay in one of the nine participating mixed medical/surgical intensive care units ≥24 hours; the CONSORT trial diagram is displayed in supplementary figure 1. Patients were randomized 1:1 either to treatment according to international guidelines: 'standard exposure arm', or to same guidelines but supplemented with daily drug-escalation initiated upon procalcitonin increases ('high exposure'-arm); 28-day mortality was 31.8% and comparable between the two groups, as reported[13].

To be eligible, patients had to be ≥18 years, enrolled within 24 hours of admission to the intensive care unit and have an expected intensive care-admission length of ≥ 24 hours. Patients with known bilirubin >40 mg/dL and triglycerides >1000 mg/dL (not suspensive) were not eligible (interference with procalcitonin measurements), as were patients who were judged to be at an increased risk from blood sampling. The inclusion criteria were broad since infection is frequent and often causes complications in the patient group and to increase the external validity of the results. The person or next of kin gave informed consent. The study protocol was approved by the regional ethics

committees in Denmark (H-KF-272-753) and adheres to the Helsinki declaration, revised in Seoul 2008.

In the present analyses we explored presence and duration of renal failure as well as change in renal function during the observed time. Endpoints are defined in *statistical analysis* below. Patients were followed until day 28. The primary trial protocol and the analysis plan is available in the online supplement. Analysis was by intention to treat: NCT00271752.

Randomization and masking

Randomization was performed 1:1 using a computerized algorithm created by the database manager (JK) with concealed block-size, pre-stratified for site of recruitment, initial APACHE-II and age (entered in an encrypted screening form in a password protected website); investigators were masked to assignment before, but not after, randomization. All investigators were trained by the coordinating centre and had to register in an investigator-database. Investigators, treating physicians and the coordinator were unaware of outcomes during the study, as were they of all procalcitonin measurements in the 'standard exposure' (control)-group.

Antibiotic therapy in the two arms

The investigators enrolled participants and assigned the 'high exposure group' participants to the intervention. In the 'standard exposure' group, the antimicrobial treatment was guided according to current clinical guidelines[14], based on clinical assessment, microbiology and radiology among other parameters, as described elsewhere[13]

In the 'high exposure' group, the use of antimicrobial interventions was guided by the same clinical guidelines as in the 'standard exposure' group to ascertain the best standard of care therapy for all patients, and additionally antimicrobial interventions were initiated whenever procalcitonin levels were not decreasing at a pre-defined pace (supplementary figure 2) and diagram D1 in the online supplement where a site-adjusted local guideline is displayed.

Measurements, data collection and follow-up

Blood samples for biomarker measurement were made daily in the intensive care unit, beginning immediately after randomization. The assay used was the Kryptor®-PCT. Organ failure and antibiotic exposure was followed up for until 28 days or death, as described[13]. Mortality was followed via the National Patient Register in which all deaths in Denmark are registered within 14 days. Good Clinical Practice guidelines were applied. The regional ethics board approved the protocol (H-KF-01-272-753).

Statistical analysis

The primary endpoint was 'estimated GFR<60 ml/min/1.73 m^2' and several analyses were made to explore this: 'days with estimated GFR<60 ml/min/1.73 m^2', 'risk of estimated GFR<60 ml/min/1.73 m^2 on day 1-7'. Secondary endpoints were a) delta eGFR after starting/stopping a drug, b) RIFLE-criteria *Risk* 'R', *Injury* 'I' and *Failure* 'F' www.adqi.net. ~~Analyses for renal failure endpoints were divided into: I) dichotomous endpoints to explore whether renal failure emerged during therapy with the investigated antibiotics and II) quantitative endpoints to explore whether existing renal failure was prolonged during therapy. Dichotomous endpoints were: 1) RIFLE-criteria 'R', 'I' and 'F' www.adqi.net, 2) 'ever' eGFR<30 or 60 ml/min/1.73m^2~~, Other endpoints explored were ~~3)~~ 'ever' blood-urea level ≥20 mmol/L and eGFR<30. ~~Quantitative endpoints were based on the time lived with eGFR<30 or 60 ml/min/1.73m^2 and the day-to-day change in eGFR.~~

The multiple effects eGFR 'slope' analyses, were adjusted for the following variables: treatment arm ('high exposure' vs. 'standard exposure'), age (≥65 vs. <65 years), gender, baseline APACHE II score (≥20 vs. <20), degree of host response/infection at baseline (severe sepsis/septic shock vs. milder or no infection as defined[15]), the eGFR at initiation of the investigated antibiotic, and finally, whether the patient at baseline was considered to be 'surgical' or 'medical'.

Comparisons were made between treatment arms using Students t-tests (for normal distributed continuous data) and Mann-Whitney U-tests (for non-normally distributed continuous data). Chi-squared tests and logistic regression models were used to test categorical variables. Time-to-event analyses comparing the 'high exposure' group with the 'standard exposure' group were performed using Kaplan-Meier plots and Cox proportional hazards models. Interactions were explored whenever an interaction could be rationally expected according to background literature, for the multivariate models performed. Statistical analyses were performed using STATA Version 10.2, and SAS version 9.1. All reported p-values are 2-sided using a level of significance of 0.05.

Sample size

~~For the present hypothesis, two sample size calculations were performed; one for a chi-square for equal proportions analysis for the originally randomized arms, and one for a multivariable logistic regression analysis, both with a limit for type I error of 5% and a power to avoid type II error of 80%. For the chi-square analysis, using a premise of the endpoint occurring in 20% of patients in the 'standard exposure' group and with 1200 patients randomized, a detection limit (one-sided) for relative risk of 1.3 in the 'high exposure' group was established.~~A ~~For the~~ multivariate approach power calculation was made:~~, t~~The summed squared correlations (Σrho^2) to the risk of the antibiotic drug investigated, was set to 0.3. The frequency of the endpoint in the 'standard exposure' group was set to 20%~~ and~~, the sample size was set to 1200, ~~were set as for the chi-square analysis~~ and the frequency of the exposure was set at 30%, which resulted in a detection limit for odds ratio of ≥1.5 (or ≤0.67).

Results

Baseline characteristics

Nine sites included 1200 persons between 09/01/06 and 02/06/09. Eighty-three percent of the patients were assessed by the investigator to have an infection at baseline and 81% of the patients suffered from chronic co-morbidity. Supplementary table 1~~Table 1~~ briefly summarizes baseline characteristics. Mortality was comparable between the two groups, as reported[13].

Follow-up

Follow-up for renal measures during the 28-day study period was made on 9,348 days in the 'standard-exposure' group of 10,755 days alive and admitted to hospital (86.9%) vs. 9,866 of 11,380 days in the 'high exposure group' (86.7%). If time after discharge from hospital (where no S-creatinine values were determined) until day 28 was included, the percentage of days with assessment of renal failure was 71.2% (9,348/13,130 days) vs. 73.8% (9,866/13,377 days)."

Use of Antibiotics

The antibiotics used most while admitted to the ICU were piperacillin/tazobactam, cefuroxim, meropenem and ciprofloxacin, and there was a substantial higher use of piperacillin/tazobactam and ciprofloxacin in the 'high exposure' arm (supplementary table 2~~table 2~~). Vancomycin was used to a lesser extent in both groups and aminoglycosides and colistin were used rarely in both groups. The median length of an antibiotic course was prolonged using the 'high exposure'-algorithm (6 days (IQR 3, 11) vs. 4 days (IQR 3, 10), p=0.004.

Renal failure in the originally randomized study arms

The % of days within day 1-28 with eGFR≤ 60 ml/min/m^2 was 48% in the 'high exposure' arm vs. 43% in the 'standard exposure' arm, p<0.0001. Results in table 1~~3~~ are estimated eGFR values, based on actual measured S-creatinine values; results regarding days with eGFR were comparable if

using the 'last observation carried forward' approach (not shown). RIFLE-criterion 'R' occurred more often within day 1-28 in the 'high exposure' arm than the 'standard exposure' arm: 209 patients vs. 170 patients, p=0.02, as did blood urea levels exceeding 20 mmol/L: 253 (43.4%) vs. 217 (37.4%), p=0.04.

The frequency of renal failure on the last day of follow-up was comparable between the arms (table 2), underlining that the results depicted in table 1~~3~~ reflect a temporary extension of duration of renal failure in the "high exposure group" and furthermore that this observation is not explained by premature discharge of renally incompetent patients in the 'standard exposure' arm.

Glomerular Filtration Rate changes and exposure to certain antibiotics

Comparison of the eGFR of all patients (both study arms) for the first ten days after starting on the most frequently used betalactam antibiotics showed that the slowest recovery of renal function was observed in patients on piperacillin/tazobactam as compared to patients on meropenem or cefuroxim (figure 1~~3~~). A multiple effects model investigating the eGFR regression coefficient ('increase in eGFR') per day on these drugs confirmed that renal recovery was lowest in patients on piperacillin/tazobactam (~~figure 4~~table 3). Of note, renal recovery seems to be low in patients exposed to cefuroxim, but as displayed in fig. 1~~3~~, this drug is given to patients with a relatively normal renal function (leaving few possibilities for 'recovery').

For the first five days following discontinuation of these drugs, adjusting for the same variables, eGFR increased at the highest rate in patients receiving ~~,~~ piperacillin/tazobactam (table 3). ~~, 2.7 ml/min/1.73 m^2 [95% CI: 2.3 – 3.1 ml/min/1.73 m^2]); meropenem, 0.2 ml/min/1.73 m^2 [0.5 – 0.9], cefuroxim, 0.0 ml/min/1.73 m^2 [0.4 – 0.4],~~

The frequency of eGFR<60 ml/min/1.73 m^2 on day 7 (or at death or last follow-up day) in the trial was 523/1200 = 43.6%. This endpoint was investigated in a forward censored (p<0.1) logistic

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regression. As a sensitivity analysis a logistic regression model with forward censoring of variables was built, where the endpoint was 'eGFR<60 ml/min/1.73 m^2 at day seven from study entry'. Variables were included if they were associated with the endpoint with p<0.1). Patients who died or who were discharged from hospital before day seven were counted with their last eGFR measurement. Use of piperacillin/tazobactam and other frequently used beta-lactam drugs for at least three days within these first seven days, as well as known and suspected predictors of renal failure were explored in a multivariable logistic regression analysis. Five independent predictors of renal failure on day 7 were identified: Age above 65 years, APACHE II score >20, Charlson´s co-morbidity score ≥2, estimated GFR at baseline and use of piperacillin/tazobactam for at least 3 days within the first 7 days (table 4) for at least three days within these first seven days was found to be an independent predictor of eGFR<60 ml/min/1,73 m^2 at day seven (OR: 1.6 [95% CI: 1.1 – 2.4]), whereas treatment with cefuroxim (OR: 1.2 [95% CI: 0.8 – 1.8]) or meropenem (OR: 0.9 [95% CI: 0.5 – 1.4]) for three days or more were not predictors of this endpoint. The following modifications did not alter the signal of this analysis: 1) eExcluding all patients who died within the first seven days, 2) excluding all patients with invasive fungal infection on day 1-28, 3) combining the betalactam exposure with exposure to flour-quinolone exposure (data not shown) or 4) adding 'Alert-procalcitonin' at baseline as a variable, did not alter the signal (data not shown).

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Discussion

Principal findings

We observed that the duration of renal failure is prolonged in critically ill patients randomized to receive high exposure to broad-spectrum antibiotics and escalated diagnostic work-up according to a biomarker-strategy, compared to patients randomized to receive standard care according to guidelines regarding use of antibiotics and diagnostics. This difference in renal function was mainly

confined to a prolongation of existing renal dysfunction, since there was only a moderate, although significant, difference in de novo acute renal failure.

To our knowledge, this study provides the first clinical ~~substantive~~ report ~~evidence~~ to inform this critical issue within ICU medicine. Firstly, the study was a randomized, good clinical practice controlled trial with a high sample size for comparison of organ failure, and the patients' baseline characteristics in general and specifically regarding renal parameters, were comparable. Secondly, the rate of follow-up, although not complete for the entire period, was high and equal among the groups and the rate of renal failure on the last day of follow-up in the two groups was comparable. Thus, the observed increased risk of persistent renal failure in the "high-exposure group" is attributable to this intervention in some way.

The intervention consisted of an increased number of culture samples, a proposed initiative to do further diagnostic imaging (no observed difference) and a rapid and aggressive antibiotic escalation with certain drugs, which was documented to be of substantial extent (supplementary table 2). As a moderate increase in microbiologic sampling would not cause renal failure, and since there was no observed increase in diagnostic imaging, these interventions seems implausible reasons to explain the observations depicted in table 1~~3~~.

This leaves us with the documented ~~(table 2)~~ escalation in use of piperacillin/tazobactam and ciprofloxacin as possible explanations. Before concluding, that the observed renal dysfunction was caused directly by one (or both) of these drugs, we wanted to exclude the possibility that the results had appeared because of a derived effect of an increase in fungal infections. Fungal infections have been linked to broad-spectrum antibiotics[16], and renal failure is a well-known complication to some antifungals[17]. However, excluding all patients with invasive fungal infections did not alter the results.

Based on these results, and after having excluded other potential explanations, we realized

that nephrotoxicity from piperacillin/tazobactam and/or ciprofloxacin was the most plausible explanation of the observed renal dysfunction. To further substantiate this, several analyses were conducted. A multiple effects model was built to examine the GFR in the days after administration of different frequently used drugs. This model included the five most often administered antibiotics, including piperacillin/tazobactam, meropenem, cefuroxim, ciprofloxacin and vancomycin along with other known and suspected causes of renal failure. In this model, the use of piperacillin/tazobactam was associated with a striking low rate of GFR-improvement, compared to the other drugs investigated. Intriguingly, this adverse effect appears to be reversible, since patients in whom, piperacillin/tazobactam was discontinued, had the fastest improvement in renal function as compared with patients on other antibiotic courses. Several sensitivity analyses were performed with findings consistent with this observation.

Comparison with other studies

Although clinical evidence regarding renal failure according to use of piperacillin/tazobactam in ICU patients has been limited, the influence of piperacillin on renal function has been investigated in healthy volunteers in laboratory experiments. In a cross-over experiment, the influence on drug clearance from concurrent administration of piperacillin and flucloxacillin was estimated[18]. The authors observed that flucloxacillin clearance was reduced to 45% [90% CI: 40 – 50%] when piperacillin was administered simultaneously, whereas piperacillin clearance was unaffected by concurrent flucloxacillin administration. Time-clearance slope modeling identified competitive inhibition of renal tubular secretion as the most likely explanation. Piperacillin-induced reduction of imipenem clearance[19] and of tazobactam clearance has also been found[20], and a high correlation between creatinin clearance and piperacillin clearance has been documented[21], and thus, it is plausible that piperacillin specifically causes nephrotoxicity.

Additionally, the published randomized trials comparing piperacillin/tazobactam with other beta-lactam drugs in intensive care unit settings are scarce, underpowered for assessment of renal failure endpoints and do generally not address renal endpoints[5-7]. Trials from other settings: haematological patients, diabetes patients, and surgical settings do generally not investigate renal failure endpoints, and in the few (non-ICU) trials that do report kidney endpoints, the total frequency of these makes the power to avoid type II error very low (diagram D2, online ~~digital~~ supplement).

Strengths and weaknesses of the study

Although our study is performed on analyses from a large randomized good clinical practice controlled trial with a stringent methodology and a high level of follow-up, there are limitations that deserve mentioning: First, follow-up for organ-related measures was not complete, although we followed patients for all blood samples done in 1) the hospital, at which they were initially recruited, 2) other hospitals in Denmark, where we had electronic access to blood samples. However, patients who continued to suffer from renal failure when discharged from hospital, were out of reach for follow-up for their renal function. Of note, the fraction of patients with remaining renal failure at time of discharge was comparable between the two groups (table 24), and hence it is unlikely that this lack of ability to ascertain renal outcome contributed to our main findings.

Second, eGFR may not be an accurate measure of creatinine clearance, as recently documented by Martin et al. [22]. However, even though this measure is not accurate to describe the creatinine clearance, changes in eGFR reflect changes in renal function, as validated, and is closely correlated to outcome[23]. Additionally, we found that eGFR<60 ml/min/1.73 m^2 on day 7 is a strong independent predictor of mortality.

~~Second~~Third, the study was a post hoc analysis using a previously published trial as material. We have tried to compensate for this by writing a detailed analysis-plan based on the hypotheses, we

wanted to test, before analysis. Third, although the sample size was relatively large compared to most other randomized trials in this setting, the sample size for these secondary analyses were based on the assumption of 25% renal failure in the 'standard exposure group' and a relative risk of 1.25 in the 'high exposure group'. The observed numbers were 21% and 1.22 which calls for a slightly higher sample size. However, the sample size needed to show the differences observed in the multivariable analyses was far smaller, and since these analyses confirmed the main findings, we do not think the results are due to chance.

In this trial, for the first time ever to our knowledge, random allocation to high exposure to broad-spectrum antibiotics in the intensive care unit has been systematically applied according to a systematic ~~randomized~~ algorithm and this resulted in prolongation of renal failure. The results were confirmed when excluding patients with fungal infections, and a multiple effects model revealed a particularly low renal recovery in patients while piperacillin/tazobactam was administered and a remarkable recovery when discontinuing this drug; a finding that was specific for this drug. Several other crude and adjusted models likewise confirmed the findings. Finally, the results from this trial are supported by human experimental studies.

Conclusion

In conclusion, the use of piperacillin/tazobactam caused a delayed renal recovery in critically ill patients, and renal function improved after discontinuation of the drug. However, the study is not designed to ~~-~~investigate ~~-~~d*e novo* emergence of renal failure, since the lowest renal function is at baseline in most patients. We cannot within the sample size and follow-up time of this trial establish whether the use of piperacillin/tazobactam, in some cases cause~~s~~ persistent renal failure, and thus, further research to explore this is warranted. We think this impact on renal function is more likely caused by a toxic effect on the renal tubule than by a lack of effect towards the infection, since this

drug is independently associated with a high chance of survival in other infected populations[8], and we must emphasize that our findings are strictly confined to critically ill patients.

Contributors

JUJ designed the study, made the data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, and drafted and the paper. He is guarantor. JUJ, ZF and JK cleaned and analysed the data. JL, BL, LH, MHB, TM, MHA, KJT, JL, MS, HT, PS-J, AØL, DGS, NR, KT, PCF, KML, NED, MEJ, LR, CØ, ZF, JK and JG made input study design, data collection tools and analysis plan and on the manuscript. JUJ implemented the trial at the centers. All members of the Procalcitonin And Survival Study (PASS) Group assisted in designing the trial. The members of the PASS study group are as follows: Central Coordinating Centre - J.U. Jensen, B. Lundgren, J. Grarup, M.L. Jakobsen, S. S. Reilev, M. Kofoed-Djursner, J. D. Lundgren; Regional Coordinating Centres - Hvidovre - J. Løken, M. Steensen; Gentofte - T. Mohr, K. Thornberg, K. Thormar; Hillerød - L.Hein, M. Bestle; Glostrup - D. Strange, A.Ø. Lauritsen; Herlev - H. Tousi, P. Søe-Jensen; Roskilde - N. Reiter, N.E. Drenck; Skejby - M.H. Andersen, P. Fjeldborg; Århus - K.M. Larsen; Data Management & Statistical Centre - Z. Fox, J. Kjær, D. Kristensen; Procalcitonin Analysis & Logistics Centre - J.U.Jensen, B. Lundgren, M. B. Rasmussen, C. S.v.Hallas, M. Zacho, J. Iversen, T. Leerbeck, M. Jeppesen, K.S. Hansen, K.B. Jensen; Data and Safety Monitoring Board - H. Masur (Chair), J. Chastre, H. Schønheyder, C. Pedersen; Clinical Microbiology Management – B. Lundgren, J. D. Knudsen, A. Friis-Møller, K. Schønning, A. Lester, H. Westh, G. Lisby, J.K. Møller, B. Bruun, J.J. Christensen, C. Østergaard, M. Arpi, K. Astvad, M.D. Bartels, J. Engberg, H. Fjeldsøe-Nielsen, U.S. Jensen; PASS Site Clinical Investigators (numbers of recruited persons are in parentheses) - Glostrup (290) – L. Hein, T. Mohr, D. G. Strange, P. L. Petersen, A. Ø. Lauritsen, S. Hougaard, T. Mantoni, L. Nebrich, A. Bendtsen, L.H. Andersen, F. Bærentzen, Andreas Eversbusch, B. Bømler, R. Martusevicius, T.

Nielsen. P.M. Bådstøløkken, C. Maschmann, U. Grevstad, P. Hallas, A. Lindhardt, T. Galle, K. Graeser, E. Hohwu-Christensen, P. Gregersen, H.C. Boesen, L.M. Pedersen, K. Thiesen, L.C. Hallengreen, I. Rye, J. Cordtz, K.R. Madsen, P.R.C. Kirkegaard, L. Findsen, L.H. Nielsen, D.H. Pedersen, J.H. Andersen, C. Albrechtsen, A. Jacobsen, T. Jansen, A.G. Jensen, H.H. Jørgensen, M. Vazin; Gentofte (209) – L. Lipsius, K. Thornberg, J. Nielsen, K. Thormar, M. Skielboe, B. Thage, C. Thoft, M. Uldbjerg, E. Anderlo, M. Engsig, F. Hani, R.B. Jacobsen. L. Mulla, U. Skram; Herlev (154) – H. Tousi, P. Søe-Jensen, T. Waldau, T. Faber, B. Andersen, I. Gillesberg, A. Christensen, C. Hartmann, R. Albret, D.S. Dinesen, K. Gani, M. Ibsen; Hvidovre (148) – J. Løken, M. Steensen, J.A. Petersen, P. Carl, E. Gade, D. Solevad, C. Heiring, M. Jørgensen, K. Ekelund, A. Afshari, N. Hammer, M. Bitsch, J.S. Hansen, C. Wamberg, T.D. Clausen, R. Winkel, J. Huusom, D.L. Buck, U. Grevstad, E. Aasvang, K. Lenz, P. Mellado, H. Karacan, J. Hidestål, J. Høgagard, J. Højbjerg, J. Højlund, M. Johansen, S. Strande; Hillerød (138) – M. Bestle, S. Hestad, M. Østergaard, N. Wesche, S.A. Nielsen, H. Christensen, H. Blom, C.H. Jensen K. Nielsen, N.G. Holler, K.A. Jeppesen; Århus-Skejby (94) – M.H. Andersen, P. Fjeldborg, A. Vestergaard, O. Viborg, C.D. Rossau; Roskilde (90) – N. Reiter, M. Glæemose, M.B.Wranér, C.B. Thomsen, B. Rasmussen, C. Lund-Rasmussen, B. Bech, K. Bjerregaard, L. Spliid, L.L.W. Nielsen, N.E. Drenck; Århus-Centre (63) – K.M. Larsen, M. Goldinger, D. Illum, C. Jessen, A. Christiansen, A. Berg, T. Elkmann, J.A.K. Pedersen, M. Simonsen; Bispebjerg (14) H. Joensen, H. Alstrøm, C. Svane, A. Engquist.

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Competing interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that the trial was funded mainly by the Danish State (Danish Research Council) and : all authors state that they have no relationships with companies that might have an interest in the submitted work in the previous 3 years; their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and all authors have no non-financial interests that may be relevant to the submitted work.

Ethical approval

The study was approved by the ethics committee for Copenhagen and Frederiksberg community (now Ethics Committee for the Capitol Region): H-KF-01-272-753. Patient consent: We received written consent from the patient or the next of kin for trial inclusion.

Data sharing

No additional data available.

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Kidney failure related to broad-spectrum antibiotics in critically ill patients: secondary end point results from a 1200 patient randomized trial

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Complete List of Authors:	Jensen, Jens-Ulrik; Faculty of Health Sciences, University of Copenhagen, Copenhagen HIV Programme; University Hospital of Copenhagen, Bispebjerg, Pulmonary Medicine (L) Hein, Lars; Copenhagen University Hospital Hillerød, Department of Anesthesia and Intensive Care ; Copenhagen University Hospital Glostrup, Department of Anesthesia and Intensive Care Lundgren, Bettina; University Hospital of Copenhagen Rigshospitalet, Diagnostic Centre Bestle, Morten; Copenhagen University Hospital Hillerød, Department of Anesthesia and Intensive Care Mohr, Thomas; Copenhagen University Hospital Gentofte, Department of Anesthesia and Intensive Care Andersen, Mads; Aarhus University Hospital in Skejby, Department of Anesthesia and Intensive Care Thornberg, Klaus; Copenhagen University Hospital Gentofte, Department of Anesthesia and Intensive Care Løken, Jesper; Copenhagen University Hospital Hvidovre, Department of Anesthesia and Intensive Care Steensen, Morten; Copenhagen University Hospital Hvidovre, Department of Anesthesia and Intensive Care Fox, Zoë; Royal Free Hospital School of Medicine in London, Research Department of Infection and Population Health; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Tousi, Hamid; Copenhagen University Hospital Herlev, Department of Anesthesia and Intensive Care Søe-Jensen, Peter; Copenhagen University Hospital Herlev, Department of Anesthesia and Intensive Care Lauritsen, Anne; Copenhagen University Hospital Glostrup, Department of Anesthesia and Intensive Care Strange, Ditte; Copenhagen University Hospital Glostrup, Department of Anesthesia and Intensive Care Reiter, Nanna; University Hospital in Roskilde, Department of Anesthesia and Intensive Care Thormar, Katrin; Copenhagen University Hospital Gentofte, Department of Anesthesia and Intensive Care Fjeldbord, Paul; Aarhus University Hospital in Skejby, Department of Anesthesia and Intensive Care Larsen, Kim; Aarhus University Hospital in Aarhus, Department of Anesthesia and Intensive Care Drenck, Niels-Erik; University Hospital in Roskilde, Department of

	Anesthesia and Intensive Care Østergaard, Christian; Copenhagen University Hospital Hvidovre, Clinical Microbiology Johansen, Maria; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Nielsen, Lene; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Kjær, Jesper; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Grarup, Jesper; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme Lundgren, Jens; University of Copenhagen, Faculty of Health Sciences, Copenhagen HIV Programme; Copenhagen University Hospital Rigshospitalet, Infectious Diseases
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Kidney failure related to broad-spectrum antibiotics in critically ill patients: secondary end point results from a 1200 patient randomized trial

Corresponding author Jens-Ulrik Jensen, Copenhagen HIV Programme, The Panum Institute, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen N, juj@cphiv.dk

Jens Ulrik Jensen *medical doctor*[1,2], Lars Hein *anaesthetist*[3,4], Bettina Lundgren *centre director, hospital diagnostic centre* [2,5], Morten Heiberg Bestle *anaesthetist* [4], Thomas Mohr *anaesthetist*[6], Mads Holmen Andersen *anaesthetist*[7], Klaus Julius Thornberg *anaesthetist*[6], Jesper Løken *anaesthetist*[8], Morten Steensen *anaesthetist*[8], Zoe Fox *biostatistician*[1,9], Hamid Tousi *anaesthetist*[10], Peter Søe-Jensen *anaesthetist*[10,] Anne Øberg Lauritsen *anaesthetist*[3], Ditte Gry Strange *anaesthetist*[3], Nanna Reiter *anaesthetist* [11], Katrin Thormar *anaesthetist*[6], Paul Christian Fjeldborg *anaesthetist* [7], Kim Michael Larsen *anaesthetist*[12], Niels-Erik Drenck *anaesthetist*[11] Maria Egede Johansen *junior research associate*[1], Lene Ryom *junior research executive*[1], Christian Østergaard *senior research executive*[2,13], Jesper Kjær *database manager*,[1] Jesper Grarup *administrative leader* [1], Jens D. Lundgren *professor of infectious diseases*,[1,14] of the The Procalcitonin And Survival Study (PASS) Group*.

[1]Copenhagen HIV Programme at the University of Copenhagen; [2]Department of Clinical Microbiology at Copenhagen University Hospital Hvidovre; [3]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Glostrup; [4]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Hillerød; [5]Diagnostic Centre at Copenhagen University Hospital Rigshospitalet; [6]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Gentofte; [7]Department of Anesthesia and Intensive Care at Aarhus University Hospital in Skejby; [8]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Hvidovre; [9]Royal Free Hospital School of Medicine in London; [10]Department of

Anesthesia and Intensive Care at Copenhagen University Hospital Herlev; [11]Department of Anesthesia and Intensive Care at Copenhagen University Hospital in Roskilde; [12]Department of Anesthesia and Intensive Care at Aarhus University Hospital in Aarhus; [13]Department of Clinical Microbiology at Copenhagen University Hospital Herlev; [14]Department of Infectious Diseases at Copenhagen University Hospital Rigshospitalet. All except[9] are from Denmark. [9] is from England.

*Participating investigators are listed in the appendix.

Running Title: Broad-Spectrum Antibiotics and Renal Failure in Critically Ill Patients

Keywords: Antibiotics – Renal Failure – Sepsis – Intensive Care

Abstract

Objectives: To explore whether a strategy of more intensive antibiotic therapy leads to emergence or prolongation of renal failure in intensive care patients.

Design: Secondary analysis from a randomized antibiotic strategy trial (the PASS study). The randomized arms were conserved from the primary trial for the main analysis.

Setting: Nine mixed surgical/medical intensive care units across Denmark.

Participants: 1200 adult intensive care patients, 18+ years, expected to stay +24 hours. Exclusion criteria: Bilirubin >40 mg/dL. Triglycerides >1000 mg/dL, Increased risk from blood sampling, pregnant/breast feeding and psychiatric patients.

Interventions: Patients were randomized to: guideline-based therapy ('standard-exposure'-arm), or to guideline-based therapy supplemented with antibiotic escalation whenever procalcitonin increased on daily measurements ('high-exposure'-arm).

Main outcome measures: Primary endpoint: estimated GFR<60 ml/min/1.73 m2. Secondary endpoints: a) delta eGFR after starting/stopping a drug, b) RIFLE criterion *Risk* "R", *Injury* 'I' and *Failure* 'F'. Analysis was by intention to treat.

Results: 28-day mortality was 31.8% and comparable (Jensen et al, CCM 2011). A total of 3672/7634 (48.1%) study days during follow-up in the 'high-exposure' vs. 3016/6949 (43.4%) in the 'standard-exposure'-arm were spent with eGFR <60 ml/min/1.73m2, p<0.001. In a multiple effects model, piperacillin/tazobactam was identified as causing the lowest rate of renal recovery of all antibiotics: 1.0 ml/min/1.73 m2 per 24h while exposed to this drug [95% CI: 0.7 – 1.3 ml/min/1.73 m2/24h] vs. meropenem: 2.9 ml/min/1.73 m2/24h [2.5 – 3.3 ml/min/1.73 m2/24h]); after discontinuing piperacillin/tazobactam, the renal recovery rate increased: 2.7 ml/min/1.73 m2 /24h [2.3 – 3.1 ml/min/1.73 m2 /24h]). eGFR<60 ml/min/1.73m2 in the two groups at entry and at last day of follow-up was 57% vs. 55% and 41% vs. 39%, resp.

Conclusions: Piperacillin/tazobactam was identified as a cause of delayed renal recovery in critically ill patients. This nephrotoxicity was not observed when using other beta-lactam antibiotics.

Trial registration ClinicalTrials.gov identifier: NCT00271752.

Introduction

Frequent complications to sepsis are organ failure, especially respiratory failure and renal failure [1-3]. Critically ill patients are more vulnerable to organ-related drug toxicities than less severely ill patients[4]. Randomized trials assessing safety of broad-spectrum antibiotics in intensive care settings are generally scarce, do not have sufficient statistical power for assessing organ failure endpoints, and do often not include defined kidney organ failure endpoints[5-7]. Data on renal failure endpoints are also sparse in the published trials from other patient populations, and since the absolute risk of renal failure is low for these patients, analyses may likely have been underpowered[8-12].

To our knowledge, randomized trials comparing 'high exposure' vs. 'standard exposure to antibiotics' and specifically addressing whether these interventions affect the occurrence and duration of kidney failure have not been done before in intensive care settings.

In this secondary analysis from a randomized trial, the PASS study[13], we aimed to explore whether a strategy of more intensive antibiotic therapy leads to adverse renal outcomes within 28 days after recruitment.

In our study population (and often in severely infected ICU patients), a bacterial hit has resulted in acute onset renal failure, and this bacterial hit (and related organ failure) is often the reason for ICU admittance. In such situations, with the correct treatment of the underlying infection, we expect renal function to recover. "Lack of recovery" is a non-desirable situation, which may be very serious for the patient. We wanted to explore this, and realizing, RIFLE/AKIN could not capture

this, we have used eGFR<60 ml/min/1.73 m^2 as the primary endpoint and examined this from different angles (eGFR<60 ml/min/1.73 m^2 at day 7, days with ml/min/1.73 m^2 . The multiple effects model was built to capture actual estimates of renal function improvement using different antibiotics and adjusting for other known or suspected causes of renal dysfunction.

Secondly, if renal failure was observed from the 'high exposure' approach, to identify one or several of the antibiotics used in this trial as the cause of such a renal failure.

Methods

Trial design and participants

PASS is a multicentre randomized controlled trial in Denmark 2006-9 in 1200 adult critically ill patients, expected to stay in one of the nine participating mixed medical/surgical intensive care units ≥24 hours; the CONSORT trial diagram is displayed in supplementary figure 1. Patients were randomized 1:1 either to treatment according to international guidelines: 'standard exposure arm', or to same guidelines but supplemented with daily drug-escalation initiated upon procalcitonin increases ('high exposure'-arm); 28-day mortality was 31.8% and comparable between the two groups, as reported[13].

To be eligible, patients had to be ≥18 years, enrolled within 24 hours of admission to the intensive care unit and have an expected intensive care-admission length of ≥ 24 hours. Patients with known bilirubin >40 mg/dL and triglycerides >1000 mg/dL (not suspensive) were not eligible (interference with procalcitonin measurements), as were patients who were judged to be at an increased risk from blood sampling. The inclusion criteria were broad since infection is frequent and often causes complications in the patient group and to increase the external validity of the results. The person or next of kin gave informed consent. The study protocol was approved by the regional ethics committees in Denmark (H-KF-272-753) and adheres to the Helsinki declaration, revised in Seoul 2008.

In the present analyses we explored presence and duration of renal failure as well as change in renal function during the observed time. Endpoints are defined in *statistical analysis* below. Patients were followed until day 28. The primary trial protocol and the analysis plan is available in the online supplement. Analysis was by intention to treat: NCT00271752.

Randomization and masking

Randomization was performed 1:1 using a computerized algorithm created by the database manager (JK) with concealed block-size, pre-stratified for site of recruitment, initial APACHE-II and age (entered in an encrypted screening form in a password protected website); investigators were masked to assignment before, but not after, randomization. All investigators were trained by the coordinating centre and had to register in an investigator-database. Investigators, treating physicians and the coordinator were unaware of outcomes during the study, as were they of all procalcitonin measurements in the 'standard exposure' (control)-group.

Antibiotic therapy in the two arms

The investigators enrolled participants and assigned the 'high exposure group' participants to the intervention. In the 'standard exposure' group, the antimicrobial treatment was guided according to current clinical guidelines[14], based on clinical assessment, microbiology and radiology among other parameters, as described elsewhere[13]

In the 'high exposure' group, the use of antimicrobial interventions was guided by the same clinical guidelines as in the 'standard exposure' group to ascertain the best standard of care therapy for all patients, and additionally antimicrobial interventions were initiated whenever procalcitonin levels were not decreasing at a pre-defined pace (supplementary figure 2) and diagram D1 in the online supplement where a site-adjusted local guideline is displayed.

Measurements, data collection and follow-up

Blood samples for biomarker measurement were made daily in the intensive care unit, beginning immediately after randomization. The assay used was the Kryptor®-PCT. Organ failure and antibiotic exposure was followed up for until 28 days or death, as described[13]. Mortality was followed via the National Patient Register in which all deaths in Denmark are registered within 14 days. Good Clinical Practice guidelines were applied. The regional ethics board approved the protocol (H-KF-01-272-753).

Statistical analysis

The primary endpoint was 'estimated GFR<60 ml/min/1.73 m^2' and several analyses were made to explore this: 'days with estimated GFR<60 ml/min/1.73 m^2', 'risk of estimated GFR<60 ml/min/1.73 m^2 on day 1-7'. Secondary endpoints were a) delta eGFR after starting/stopping a drug, b) RIFLE-criteria *Risk* 'R'. , *Injury* 'I' and *Failure* 'F' www.adqi.net. Since we explored exposure of antibiotics from baseline and forth (and not pre-ICU), in the RIFLE definition, the baseline creatinine was used (instead of an ideal eGFR). eGFR was calculated for every day. To not let this be influenced by hydration status, the baseline weight was used, and thus the relation between se-creatinine and eGFR was a first degree function for every patient. Other endpoints explored were 'ever' blood-urea level ≥20 mmol/L and eGFR<30.

The multiple effects eGFR 'slope' analyses, were adjusted for the following variables: treatment arm ('high exposure' vs. 'standard exposure'), age (≥65 vs. <65 years), gender, baseline APACHE II score (≥20 vs. <20), degree of host response/infection at baseline (severe sepsis/septic shock vs. milder or no infection as defined[15]), the eGFR at initiation of the investigated antibiotic, and finally, whether the patient at baseline was considered to be 'surgical' or 'medical'.

Comparisons were made between treatment arms using Students t-tests (for normal distributed continuous data) and Mann-Whitney U-tests (for non-normally distributed continuous data). Chi-squared tests and logistic regression models were used to test categorical variables. Time-to-event

analyses comparing the 'high exposure' group with the 'standard exposure' group were performed using Kaplan-Meier plots and Cox proportional hazards models. Interactions were explored whenever an interaction could be rationally expected according to background literature, for the multivariate models performed. Statistical analyses were performed using STATA Version 10.2, and SAS version 9.1. All reported p-values are 2-sided using a level of significance of 0.05.

Sample size

A multivariate approach power calculation was made: The summed squared correlations (Σrho^2) to the risk of the antibiotic drug investigated, was set to 0.3. The frequency of the endpoint in the 'standard exposure' group was set to 20%, the sample size was set to 1200, and the frequency of the exposure was set at 30%, which resulted in a detection limit for odds ratio of ≥ 1.5 (or ≤ 0.67).

Results

Baseline characteristics

Nine sites included 1200 persons between 09/01/06 and 02/06/09. Eighty-three percent of the patients were assessed by the investigator to have an infection at baseline and 81% of the patients suffered from chronic co-morbidity. Supplementary table 1 briefly summarizes baseline characteristics. Mortality was comparable between the two groups, as reported[13].

Follow-up

Follow-up for renal measures during the 28-day study period was made on 9,348 days in the 'standard-exposure' group of 10,755 days alive and admitted to hospital (86.9%) vs. 9,866 of 11,380 days in the 'high exposure group' (86.7%). If time after discharge from hospital (where no S-creatinine values were determined) until day 28 was included, the percentage of days with assessment of renal failure was 71.2% (9,348/13,130 days) vs. 73.8% (9,866/13,377 days)."

Use of Antibiotics

The antibiotics used most while admitted to the ICU were piperacillin/tazobactam, cefuroxim, meropenem and ciprofloxacin, and there was a substantial higher use of piperacillin/tazobactam and ciprofloxacin in the 'high exposure' arm (supplementary table 2). Vancomycin was used to a lesser extent in both groups and aminoglycosides and colistin were used rarely in both groups.

The median length of an antibiotic course was prolonged using the 'high exposure'-algorithm (6 days (IQR 3, 11) vs. 4 days (IQR 3, 10), p=0.004.

Renal failure in the originally randomized study arms

The % of days within day 1-28 with eGFR≤ 60 ml/min/m^2 was 48% in the 'high exposure' arm vs. 43% in the 'standard exposure' arm, p<0.0001. Results in table 1 are estimated eGFR values, based on actual measured S-creatinine values; results regarding days with eGFR were comparable if using the 'last observation carried forward' approach (not shown). RIFLE-criterion 'R' occurred more often within day 1-28 in the 'high exposure' arm than the 'standard exposure' arm: 209 patients vs. 170 patients, p=0.02, as did blood urea levels exceeding 20 mmol/L: 253 (43.4%) vs. 217 (37.4%), p=0.04.

The frequency of renal failure on the last day of follow-up was comparable between the arms (table 2), underlining that the results depicted in table 1 reflect a temporary extension of duration of renal failure in the "high exposure group" and furthermore that this observation is not explained by premature discharge of renally incompetent patients in the 'standard exposure' arm.

Glomerular Filtration Rate changes and exposure to certain antibiotics

Comparison of the eGFR of all patients (both study arms) for the first ten days after starting on the most frequently used betalactam antibiotics showed that the slowest recovery of renal function was

observed in patients on piperacillin/tazobactam as compared to patients on meropenem or cefuroxim (figure 1). A multiple effects model investigating the eGFR regression coefficient ('increase in eGFR') per day on these drugs confirmed that renal recovery was lowest in patients on piperacillin/tazobactam (table 3). Of note, renal recovery seems to be low in patients exposed to cefuroxim, but as displayed in fig. 1, this drug is given to patients with a relatively normal renal function (leaving few possibilities for 'recovery').

For the first five days following discontinuation of these drugs, adjusting for the same variables, eGFR increased at the highest rate in patients receiving piperacillin/tazobactam (table 3).

The frequency of eGFR<60 ml/min/1.73 m^2 on day 7 (or at death or last follow-up day) in the trial was 523/1200 = 43.6%. This endpoint was investigated in a forward censored ($p<0.1$) logistic regression. Use of piperacillin/tazobactam and other frequently used beta-lactam drugs for at least three days within these first seven days, as well as known and suspected predictors of renal failure were explored in a multivariable logistic regression analysis. Five independent predictors of renal failure on day 7 were identified: Age above 65 years, APACHE II score >20, Charlson´s co-morbidity score ≥2, estimated GFR at baseline and use of piperacillin/tazobactam for at least 3 days within the first 7 days (table 4) Excluding all patients who died within the first seven days, excluding all patients with invasive fungal infection on day 1-28, combining the betalactam exposure with exposure to flour-quinolone exposure (data not shown) or 4) adding 'Alert-procalcitonin' at baseline as a variable, did not alter the signal (data not shown). To validate the endpoint as a predictor of mortality, a Cox regression was done; eGFR <60 mL/min/1.73 m^2 on day 7 was found to be the strongest predictor of 'all cause mortality day 7-28' of all tested variables (Table T1, supplementary material).

Discussion

Principal findings

We observed that the duration of renal failure is prolonged in critically ill patients randomized to receive high exposure to broad-spectrum antibiotics and escalated diagnostic work-up according to a biomarker-strategy, compared to patients randomized to receive standard care according to guidelines regarding use of antibiotics and diagnostics. This difference in renal function was mainly confined to a prolongation of existing renal dysfunction, since there was only a moderate, although significant, difference in de novo acute renal failure.

To our knowledge, this study provides the first clinical report to inform this critical issue within ICU medicine. Firstly, the study was a randomized, good clinical practice controlled trial with a high sample size for comparison of organ failure, and the patients' baseline characteristics in general and specifically regarding renal parameters, were comparable. Secondly, the rate of follow-up, although not complete for the entire period, was high and equal among the groups and the rate of renal failure on the last day of follow-up in the two groups was comparable. Thus, the observed increased risk of persistent renal failure in the "high-exposure group" is attributable to this intervention in some way.

The intervention consisted of an increased number of culture samples, a proposed initiative to do further diagnostic imaging (no observed difference) and a rapid and aggressive antibiotic escalation with certain drugs, which was documented to be of substantial extent (supplementary table 2). As a moderate increase in microbiologic sampling would not cause renal failure, and since there was no observed increase in diagnostic imaging, these interventions seems implausible reasons to explain the observations depicted in table 1.

This leaves us with the documented escalation in use of piperacillin/tazobactam and ciprofloxacin as possible explanations. Before concluding, that the observed renal dysfunction was caused directly by one (or both) of these drugs, we wanted to exclude the possibility that the results had appeared because of a derived effect of an increase in fungal infections. Fungal infections have been linked to broad-spectrum antibiotics[16], and renal failure is a well-known complication to some

antifungals[17]. However, excluding all patients with invasive fungal infections did not alter the results.

Based on these results, and after having excluded other potential explanations, we realized that nephrotoxicity from piperacillin/tazobactam and/or ciprofloxacin was the most plausible explanation of the observed renal dysfunction. To further substantiate this, several analyses were conducted. A multiple effects model was built to examine the GFR in the days after administration of different frequently used drugs. This model included the five most often administered antibiotics, including piperacillin/tazobactam, meropenem, cefuroxim, ciprofloxacin and vancomycin along with other known and suspected causes of renal failure. In this model, the use of piperacillin/tazobactam was associated with a striking low rate of GFR-improvement, compared to the other drugs investigated. Intriguingly, this adverse effect appears to be reversible, since patients in whom, piperacillin/tazobactam was discontinued, had the fastest improvement in renal function as compared with patients on other antibiotic courses. Several sensitivity analyses were performed with findings consistent with this observation.

Comparison with other studies

Although clinical evidence regarding renal failure according to use of piperacillin/tazobactam in ICU patients has been limited, the influence of piperacillin on renal function has been investigated in healthy volunteers in laboratory experiments. In a cross-over experiment, the influence on drug clearance from concurrent administration of piperacillin and flucloxacillin was estimated[18]. The authors observed that flucloxacillin clearance was reduced to 45% [90% CI: 40 – 50%] when piperacillin was administered simultaneously, whereas piperacillin clearance was unaffected by concurrent flucloxacillin administration. Time-clearance slope modeling identified competitive inhibition of renal tubular secretion as the most likely explanation. Piperacillin-induced reduction of imipenem clearance[19] and of tazobactam clearance has also been found[20], and a high correlation

between creatinin clearance and piperacillin clearance has been documented[21], and thus, it is plausible that piperacillin specifically causes nephrotoxicity.

Additionally, the published randomized trials comparing piperacillin/tazobactam with other beta-lactam drugs in intensive care unit settings are scarce, underpowered for assessment of renal failure endpoints and do generally not address renal endpoints[5-7]. Trials from other settings: haematological patients, diabetes patients, and surgical settings do generally not investigate renal failure endpoints, and in the few (non-ICU) trials that do report kidney endpoints, the total frequency of these makes the power to avoid type II error very low (diagram D2, online supplement).

Strengths and weaknesses of the study

Although our study is performed on analyses from a large randomized good clinical practice controlled trial with a stringent methodology and a high level of follow-up, there are limitations that deserve mentioning: First, follow-up for organ-related measures was not complete, although we followed patients for all blood samples done in 1) the hospital, at which they were initially recruited, 2) other hospitals in Denmark, where we had electronic access to blood samples. However, patients who continued to suffer from renal failure when discharged from hospital, were out of reach for follow-up for their renal function. Of note, the fraction of patients with remaining renal failure at time of discharge was comparable between the two groups (table 2), and hence it is unlikely that this lack of ability to ascertain renal outcome contributed to our main findings.

Second, eGFR may not be an accurate measure of creatinine clearance, as recently documented by Martin et al. [22]. However, even though this measure is not accurate to describe the creatinine clearance, changes in eGFR reflect changes in renal function, as validated, and is closely correlated to outcome[23]. Additionally, since hydration can be a source of error, we used the baseline weight in

the eGFR equation. Additionally, we found that eGFR<60 ml/min/1.73 m^2 on day 7 is a strong independent predictor of mortality.

Third, the RIFLE criteria used as secondary endpoint measures are not suitable to detect renal failure from baseline and forth, since the reference is defined as the pre-morbid creatinine. Hence, renal failure caused by exposure to antibiotics beginning at baseline, will not necessarily be captured using these criteria. This was the reason for not using these as primary endpoints.

Forth, the study was a post hoc analysis using a previously published trial as material. We have tried to compensate for this by writing a detailed analysis-plan based on the hypotheses, we wanted to test, before analysis. Fifth, although the sample size was relatively large compared to most other randomized trials in this setting, the sample size for these secondary analyses were based on the assumption of 25% renal failure in the 'standard exposure group' and a relative risk of 1.25 in the 'high exposure group'. The observed numbers were 21% and 1.22 which calls for a slightly higher sample size. However, the sample size needed to show the differences observed in the multivariable analyses was far smaller, and since these analyses confirmed the main findings, we do not think the results are due to chance.

In this trial, for the first time ever to our knowledge, random allocation to high exposure to broad-spectrum antibiotics in the intensive care unit has been systematically applied according to a systematic algorithm and this resulted in prolongation of renal failure. The results were confirmed when excluding patients with fungal infections, and a multiple effects model revealed a particularly low renal recovery in patients while piperacillin/tazobactam was administered and a remarkable recovery when discontinuing this drug; a finding that was specific for this drug. Several other crude and adjusted models likewise confirmed the findings. Finally, the results from this trial are supported by human experimental studies.

Conclusion

In conclusion, the use of piperacillin/tazobactam caused a delayed renal recovery in critically ill patients, and renal function improved after discontinuation of the drug. However, the study is not designed to investigate d*e novo* emergence of renal failure, since the lowest renal function is at baseline in most patients. The study was not designed to establish whether the use of piperacillin/tazobactam or other of the interventional drugs, in some cases cause persistent renal failure, and thus, further research to explore this is warranted. We think this impact on renal function is more likely caused by a – at least partially reversible - toxic effect on the renal tubule than by a lack of effect towards the infection, since this drug is independently associated with a high chance of survival in other infected populations[8], and we must emphasize that our findings are strictly confined to critically ill patients.

Contributors

JUJ designed the study, made the data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, and drafted and the paper. He is guarantor. JUJ, ZF and JK cleaned and analysed the data. JL, BL, LH, MHB, TM, MHA, KJT, JL, MS, HT, PS-J, AØL, DGS, NR, KT, PCF, KML, NED, MEJ, LR, CØ, ZF, JK and JG made input study design, data collection tools and analysis plan and on the manuscript. JUJ implemented the trial at the centers. All members of the Procalcitonin And Survival Study (PASS) Group assisted in designing the trial. The members of the PASS study group are as follows: Central Coordinating Centre - J.U. Jensen, B. Lundgren, J. Grarup, M.L. Jakobsen, S. S. Reilev, M. Kofoed-Djursner, J. D. Lundgren; Regional Coordinating Centres - Hvidovre - J. Løken, M. Steensen; Gentofte - T. Mohr, K. Thornberg, K. Thormar; Hillerød - L.Hein, M. Bestle; Glostrup - D. Strange, A.Ø. Lauritsen; Herlev - H. Tousi, P. Søe-Jensen; Roskilde - N. Reiter, N.E. Drenck; Skejby - M.H. Andersen, P. Fjeldborg; Århus - K.M. Larsen; Data Management & Statistical Centre - Z. Fox, J. Kjær, D. Kristensen; Procalcitonin Analysis & Logistics Centre - J.U.Jensen, B. Lundgren, M. B.

Rasmussen, C. S.v.Hallas, M. Zacho, J. Iversen, T. Leerbeck, M. Jeppesen, K.S. Hansen, K.B. Jensen; Data and Safety Monitoring Board - H. Masur (Chair), J. Chastre, H. Schønheyder, C. Pedersen; Clinical Microbiology Management – B. Lundgren, J. D. Knudsen, A. Friis-Møller, K. Schønning, A. Lester, H. Westh, G. Lisby, J.K. Møller, B. Bruun, J.J. Christensen, C. Østergaard, M. Arpi, K. Astvad, M.D. Bartels, J. Engberg, H. Fjeldsøe-Nielsen, U.S. Jensen; PASS Site Clinical Investigators (numbers of recruited persons are in parentheses) - Glostrup (290) – L. Hein, T. Mohr, D. G. Strange, P. L. Petersen, A. Ø. Lauritsen, S. Hougaard, T. Mantoni, L. Nebrich, A. Bendtsen, L.H. Andersen, F. Bærentzen, Andreas Eversbusch, B. Bømler, R. Martusevicius, T. Nielsen. P.M. Bådstøløkken, C. Maschmann, U. Grevstad, P. Hallas, A. Lindhardt, T. Galle, K. Graeser, E. Hohwu-Christensen, P. Gregersen, H.C. Boesen, L.M. Pedersen, K. Thiesen, L.C. Hallengreen, I. Rye, J. Cordtz, K.R. Madsen, P.R.C. Kirkegaard, L. Findsen, L.H. Nielsen, D.H. Pedersen, J.H. Andersen, C. Albrechtsen, A. Jacobsen, T. Jansen, A.G. Jensen, H.H. Jørgensen, M. Vazin; Gentofte (209) – L. Lipsius, K. Thornberg, J. Nielsen, K. Thormar, M. Skielboe, B. Thage, C. Thoft, M. Uldbjerg, E. Anderlo, M. Engsig, F. Hani, R.B. Jacobsen. L. Mulla, U. Skram; Herlev (154) – H. Tousi, P. Søe-Jensen, T. Waldau, T. Faber, B. Andersen, I. Gillesberg, A. Christensen, C. Hartmann, R. Albret, D.S. Dinesen, K. Gani, M. Ibsen; Hvidovre (148) – J. Løken, M. Steensen, J.A. Petersen, P. Carl, E. Gade, D. Solevad, C. Heiring, M. Jørgensen, K. Ekelund, A. Afshari, N. Hammer, M. Bitsch, J.S. Hansen, C. Wamberg, T.D. Clausen, R. Winkel, J. Huusom, D.L. Buck, U. Grevstad, E. Aasvang, K. Lenz, P. Mellado, H. Karacan, J. Hidestål, J. Høgagard, J. Højbjerg, J. Højlund, M. Johansen, S. Strande; Hillerød (138) – M. Bestle, S. Hestad, M. Østergaard, N. Wesche, S.A. Nielsen, H. Christensen, H. Blom, C.H. Jensen K. Nielsen, N.G. Holler, K.A. Jeppesen; Århus-Skejby (94) – M.H. Andersen, P. Fjeldborg, A. Vestergaard, O. Viborg, C.D. Rossau; Roskilde (90) – N. Reiter, M. Glæemose, M.B.Wranér, C.B. Thomsen, B. Rasmussen, C. Lund-Rasmussen, B. Bech, K. Bjerregaard, L. Spliid, L.L.W. Nielsen, N.E. Drenck; Århus-Centre

(63) – K.M. Larsen, M. Goldinger, D. Illum, C. Jessen, A. Christiansen, A. Berg, T. Elkmann, J.A.K. Pedersen, M. Simonsen; Bispebjerg (14) H. Joensen, H. Alstrøm, C. Svane, A. Engquist.

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Competing interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that the trial was funded mainly by the Danish State (Danish Research Council) and : all authors state that they have no relationships with companies that might have an interest in the submitted work in the previous 3 years; their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and all authors have no non-financial interests that may be relevant to the submitted work.

Ethical approval

The study was approved by the ethics committee for Copenhagen and Frederiksberg community (now Ethics Committee for the Capitol Region): H-KF-01-272-753. Patient consent: We received written consent from the patient or the next of kin for trial inclusion.

Data sharing

No additional data available.

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Table 1: Prevalence and duration of kidney organ failure ('Standard exposure' group vs. 'High exposure' group)

	'Standard exposure' group (N=596)	'High exposure' group (N=604)	p-value
EstimatedGFR*: N. days (% of days from day 1 to 28 with values):			
Moderately-severely impaired: (eGFR: ≤60 mL/min/1.73 m^2)	3016 (43.4%)	3672 (48.1%)	<0.0001
Severely impaired: (eGFR ≤30 mL/min/1.73 m^2)	1445 (20.8%)	1910 (25.0%)	<0.0001
Severely impaired: (eGFR ≤30 mL/min/1.73 m^2), days from day 1 to 14	984 (20.0%)	1253 (23.5%)	<0.0001
'RIFLE' criteria, N patients (%) within day 1 to 28			
'R' reached	170 (28.5%)	209 (34.6%)	0.02
'I' reached	75 (12.6%)	92 (15.2%)	0.19
'F' reached	121 (20.3%)	150 (24.8%)	0.06
'R' or death	298 (50.0%)	327 (54.1%)	0.15
'I' or death	234 (39.3%)	252 (41.7%)	0.39
'F' or death	270 (45.3%)	287 (47.5%)	0.44
Urea			
Patients with a urea level ever ≥ 20 mmol/L (day 1-28); N (%)	217 (37.4%)	253 (43.4%)	0.04

*eGFR was assessed using the Cockcroft and Gault method [Ref: Cockcroft DW, Gault MH.: Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41]. Actual measured creatinin values were used. If using the 'last observation carried forward' approach regarding creatinin measurement to take into account that patients who died in renal failure should be counted as such, did not change the signal or the statistics of these analyses. 'R':Risk, 'I': Injury, 'F': Failure. Presence of renal failure according to 'RIFLE' was assessed using the guidelines developed by the acute dialysis quality initiative (www.adqi.net)

Table 2: Prevalence of kidney organ failure on the last day of follow-up ('Standard exposure' group vs. 'High exposure' group)

	'Standard exposure' group	'High exposure' group	p-value
Survivors and patients who had last creatinine measured>24 h before death:	**(N=432)**	**(N=438)**	
Renal failure (eGFR: ≤60 mL/min/1.73 m^2)	119 (27.6%)	137 (31.3%)	0.23
Patients who died (with last creatinine measured within 24 h before death):	**(N=150)**	**(N=145)**	
Renal failure (eGFR: ≤60 mL/min/1.73 m^2)	105 (70.0%)	99 (68.3%)	0.83
All patients with creatinine measurements	**(N=582)**	**(N=583)**	
Renal failure (eGFR: ≤60 mL/min/1.73 m^2)	224 (38.5)	236 (40.5)	0.51

*eGFR was assessed using the Cockcroft and Gault method [Ref: Cockcroft DW, Gault MH.: Prediction of creatinine clearance from serum creatinine. Nephron 1976;16:31-41]. Actual measured creatinin values were used.

Table 3. Multiple effects models investigating estimated GFR changes after starting and stopping beta-lactam antibiotics

		Unadjusted analysis		Multivariable analysis	
	Variable	**Regression coefficient (95% CI)**	**P-value**	**Regression coefficient (95% CI)**	**P-value**
After starting the drug					
Piperacillin/tazobactam	Per day more on piperacillin/tazobactam	1.39 (1.17, 1.60)	<0.0001	0.99 (0.71, 1.27)	<0.0001
Meropenem	Per day more on meropenem	2.74 (2.39, 3.09)	<0.0001	2.86 (2.45, 3.28)	<0.0001
Cefuroxim	Per day more on cefuroxim	1.91 (1.67, 2.16)	<0.0001	1.27 (0.90, 1.64)	<0.0001
After stopping the drug					
Piperacillin/tazobactam	Per day after stopping piperacillin/tazobactam	2.79 (2.35, 3.24)	<0.0001	2.70 (2.26, 3.14)	<0.0001
Meropenem	Per day after stopping meropenem	0.20 (-0.51, 0.91)	0.59	0.17 (-0.52, 0.86)	0.63
Cefuroxim	Per day after stopping cefuroxim	0.13 (-0.25, 0.50)	0.51	0.01 (-0.35, 0.37)	0.96

All multivariable analyses were adjusted for: treatment arm ('low exposure' vs. 'high exposure'), gender, age (≥65 vs. <65 years), APACHE II score (≥20 vs. <20), Clinically judged infection (severe sepsis/septic shock vs. milder or no infection), patient category (surgical vs. medical) and eGFR level at administration of the antibiotic, (1: <30 ml/min/1,73 m^2, 2: 31-60 ml/min/1,73 m^2, 3: >60 ml/min/1,73 m^2).

Table 4. Multivariable logistic regression: beta-lactam antibiotics and other risk variables vs. binary endpoint eGFR<60 ml/min/1.73m^2 on day 7.

	Unadjusted analysis		Multivariable analysis	
Variable	**Odds ratio (95% CI)**	**P-value**	**Odds ratio (95% CI)**	**P-value**
Other variables				
Age (≥65 vs. <65 years)	2.36 (1.86, 3.00)	<0.0001	1.85 (1.31, 2.60)	<0.0001
APACHE II score (≥20 vs. <20)	2.49 (1.90, 3.25)	<0.0001	1.64 (1.12, 2.41)	0.01
Severe sepsis/septic shock vs. milder or no infection	2.02 (1.59, 2,56)	<0.0001	1.16 (0.82, 1.66)	0.40
Auto-immune disease (Y vs. N)	1.31 (0.73, 2.33)	0.36	NI	-
Cancer (Y vs. N)	1.26 (0.88, 1.79)	0.21	NI	-
Charlson score (≥2 vs. <2)	1.72 (1.35, 2.18)	<0.0001	1.70 (1.21, 2.40)	0.002
Surgical (Y vs. N)	1.16 (0.90, 1.50)	0.24	NI	-
Body Mass Index (≥25 vs. <25)	1.57 (1.17, 2.12)	0.003	1.19 (0.78, 1.82)	0.41
Gender (Male vs. Female)	1.25 (0.99, 1.57)	0.06	1.28 (0.92, 1.78)	0.14
eGFR level at baseline				
>60 ml/min/1,73 m^2	Ref	-	Ref	-
31-60 ml/min/1,73 m^2	14.6 (10.2, 21.0)	<0.0001	11.7 (8.0, 17.0)	<0.0001
<30 ml/min/1,73 m^2	81.1 (51.2, 128.5)	<0.0001	65.9 (40.7, 106.6)	<0.0001
Beta-lactam antibiotics				
Piperacillin/tazobactam (≥3 vs. <3 days)*	2.32 (1.82, 2.96)	<0.0001	1.70 (1.18, 2.43)	0.004
Meropenem (≥3 vs. <3 days)*	0.99 (0.71, 1.37)	0.94	NI	-
Cefuroxim (≥3 vs. <3 days)*	0.73 (0.57, 0.94)	0.01	1.24 (0.85, 1.80)	0.26

All variables entered in the multivariable analysis were adjusted for the other variables in this model. *All beta-lactam drug exposures are (≥3 vs. <3 days within the first 7 days in the study). All variables with a p-value <0.2 were included in the multivariable model. NI: Not Included.

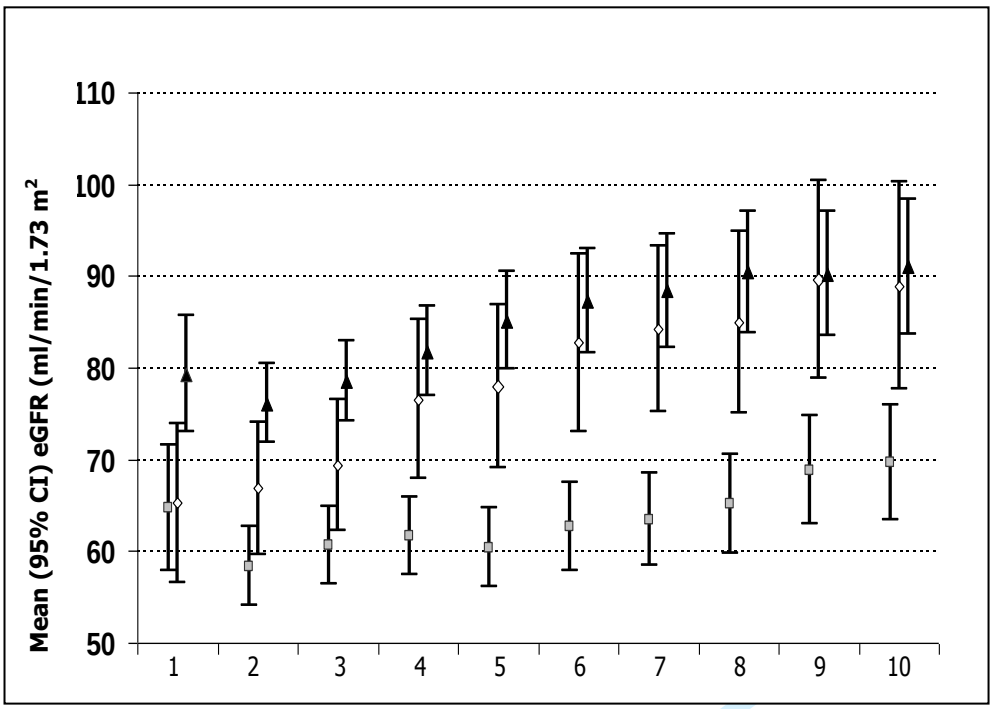
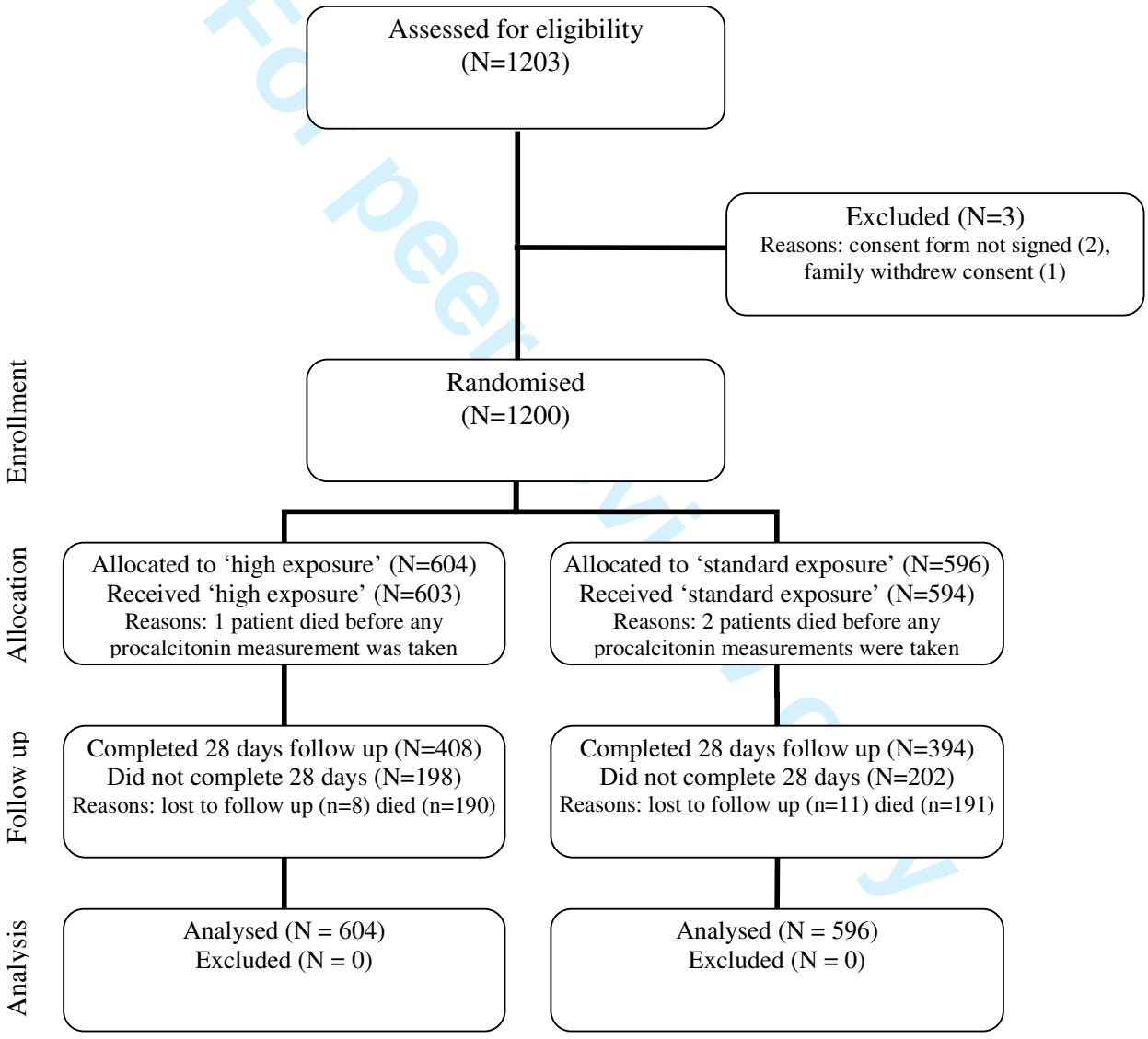
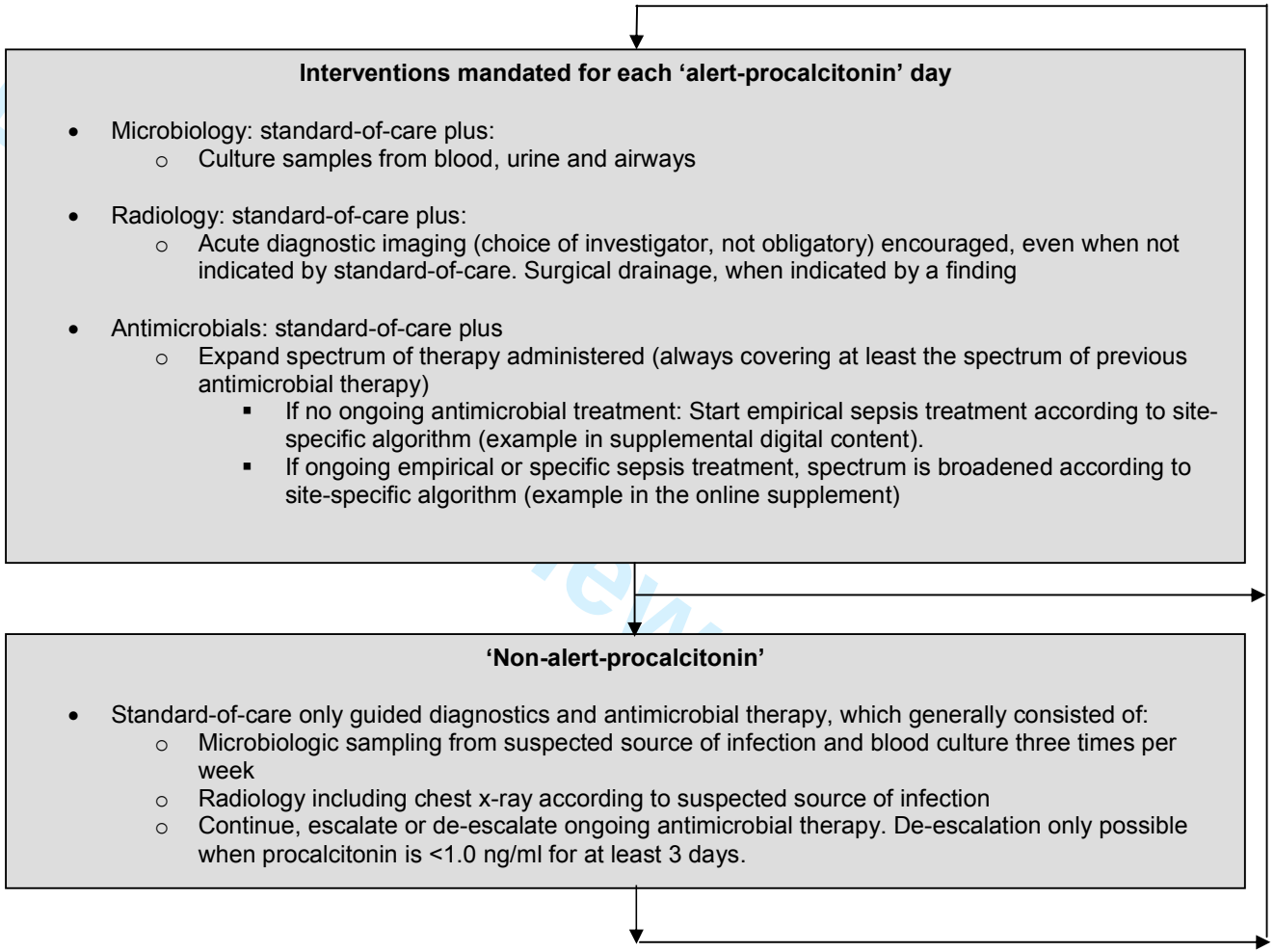


Figure 1. eGFR during ten days on cefuroxim, piperacillin/tazobactam and meropenem. ▲=cefuroxim; ▩ =piperacillin/tazobactam; ◇=meropenem.

Differences between eGFR in patients receiving piperacillin/tazobactam vs. meropenem: day 1 (p=0.78), day 2 (p=0.18), day 3 (p=0.09), day 4 (p=0.008), day 5 (p=0.001), day 6 (p=0.001), day 7 (p=0.0004), day 8 (p=0.005), day 9 (p=0.006), day 10 (p=0.02).



Supplementary Figure 1. Patient Flow Diagram of the trial


Interventions mandated for each 'alert-procalcitonin' day
Microbiology: standard-of-care plus:
Culture samples from blood, urine and airways
Radiology: standard-of-care plus:
Acute diagnostic imaging (choice of investigator, not obligatory) encouraged, even when not indicated by standard-of-care. Surgical drainage, when indicated by a finding
Antimicrobials: standard-of-care plus
Expand spectrum of therapy administered (always covering at least the spectrum of previous antimicrobial therapy)
If no ongoing antimicrobial treatment: Start empirical sepsis treatment according to site-specific algorithm (example in supplemental digital content).
If ongoing empirical or specific sepsis treatment, spectrum is broadened according to site-specific algorithm (example in the online supplement)
'Non-alert-procalcitonin'
Standard-of-care only guided diagnostics and antimicrobial therapy, which generally consisted of:
Microbiologic sampling from suspected source of infection and blood culture three times per week
Radiology including chest x-ray according to suspected source of infection
Continue, escalate or de-escalate ongoing antimicrobial therapy. De-escalation only possible when procalcitonin is <1.0 ng/ml for at least 3 days.

Supplementary Figure 1. General principles of procalcitonin-guided intervention.

At 'alert-procalcitonin' situation (≥ 1.0 ng/ml and not decreasing by at least 10% from the previous day), interventions were obligatorily conducted according to an algorithm with specific instructions for intervention, which was adapted to the antimicrobial guidelines on the site. Antimicrobials were daily adjusted according to 1) present and previous procalcitonin values, 2) infectious state of the patient (clinical presentation, microbiology, radiology etc.) and 3) history of antimicrobial use. Procalcitonin-guided antimicrobial escalation was mandatory, except when 1) there was a clear contra-indication for administering it or 2) microbiology "explaining the infectious presentation of the patient" was announced (same date) leading to specific therapy. Standard-of-Care antimicrobial diagnostics and treatment was not waived in the 'high exposure arm (nor the 'standard exposure'arm) to assure patient safety. According to the standard-of-care principle, all patients with septic shock were treated at the onset of hypotension with antimicrobials covering >95% of the causes of this condition in our hospitals. Awaiting procalcitonin results/low procalcitonin levels was not considered a plausible reason to withhold antimicrobial treatment. The treating physician was reminded daily via phone from the coordinating centre at each 'alert-procalcitonin' to intervene. In the 'standard exposure' arm, procalcitonin measurements were not available.

Supplementary Table 1. Baseline characteristics of the study participants.

	'Standard exposure' group (n=596)	'High exposure' group (n=604)	Overall (n=1200)
Age, years - median (IQR)	67 (58–75)	67 (58–76)	67 (58–76)
Male sex – no. (%)	333 (55·9%)	330 (54.6%)	663 (55.3%)
Body Mass Index, kg/m2 – median (IQR)	24.7 (22.0–27.8)	25.0 (22.5–28.7)	24.8 (22.2–27.9)
APACHE II Score - median (IQR)	18 (13–24)	18 (13–25)	18 (13–24)
Chronic co-morbidity* - no. (%)			
No chronic co-morbidities	102 (17.1)	123 (20.4)	225 (18.8)
Kidney function and electrolytes			
Creatinin, µmol/L - median (IQR)	119 (78, 197)	119 (75, 208)	119 (76, 202)
eGFR, mL/min/1.73m^2 – median (IQR)	51.4 (29.2, 80.5)	49.4 (25.4, 82.6)	50.2 (27.1, 81.5)
Carbamid, mmol/L - median (IQR)	10.3 (6.5, 17.0)	10.6 (6.3, 18.1)	10.5 (6.4, 17.4)
Na^+, mmol/l - median (IQR)	138 (134, 141)	137 (134, 141)	138 (134, 141)
K^+, mmol/l - median (IQR)	4.0 (3.7, 4.4)	4.0 (3.6, 4.5)	4.0 (3.6, 4.4)
pH - median (IQR)	7.29 (7.21–7.39)	7.29 (7.20–7.38)	7.29 (7.20–7.38)
Dialysis required, patients (%)	88 (14.8%)	86 (14.2%)	174 (14.5%)
Indicators of severity (non-renal)			
Temperature, ^{0}C - median (IQR)	37.2 (36.4–38.0)	37.3 (36.5–38.1)	37.3 (36.4–38.0)
Mean arterial pressure, mmHg - median (IQR)	71 (60–84)	72 (63–85)	71 (62–84)
Heart frequency - median (IQR)	100 (82–116)	100 (84–117)	100 (83–117)
Need for vasopressor/inotropic drug† - n (%)	315 (52.9)	326 (53.4)	641 (53.4)
Mechanical ventilation used - n (%)	401 (67.3%)	401 (66.4%)	802 (66.8%)
Biomarkers			
Alert-PCT § – no. (%)	279 (47.0)	312 (51.7)	591 (49.4)
Leukocytes, x10^9 – median (IQR)	13.0 (8.8–18.1)	12.4 (8.0–18.1)	12.8 (8.4–18.1)
C-reactive protein, mg/L – median (IQR)	131 (40–234)	137 (40–253)	135 (40–241)

Interquartile range (IQR). Acute Physiology and Chronic Health Evaluation II score (APACHE II) ranges from 0 to 71. *Chronic co-morbidity: Earlier diagnosed via hospital admission: heart failure, lung disease, cancer, diabetes, alcohol abuse, chronic infection, neurological disease, renal diseases, liver disease, gastro-intestinal disease, autoimmune disease, cancer and psychiatric disorders. †Vasopressors/inotropic drugs are considered to be epinephrine, nor-epinephrine, dopamine and dobutamine. ‡ Infections were rated according to the ACCP/SCCM definitions; investigators were trained in using them. §Alert-PCT: Procalcitonin-level not decreasing by at least 10% from the previous day and above 1.0 ng/ml. If only one measurement is available: Absolute procalcitonin-level above 1.0 ng/ml. A comprehensive baseline table is available in the primary publication from this material[13].

Supplementary Table 2. Consumption of antimicrobials during follow-up

	Standard exposure (n=596)	High exposure (n=604)	p-value
Consumption of antimicrobials			
Pip/tazo used within 28 days (DDD)	1893	2925	-
Proportion of days[a] followed where Pip/tazo was used	0.00 (0.00 – 0.33)	0.11 (0.00 – 0.56)	<0.001
Meropenem used within 28 days (DDD)	2174	2480	-
Proportion of days[a] followed where meropenem was used	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.07)	0.23
Cefuroxim used within 28 days (DDD)	4369	3390	-
Proportion of days[a] followed where cefuroxim was used	0.11 (0.00 – 0.39)	0.04 (0.00 – 0.29)	<0.001
Ciprofloxacin used within 28 days (DDD)	6210	8382	-
Proportion of days[a] followed where ciprofloxacin was used	0.21 (0.00 – 0.71)	0.33 (0.04 – 0.88)	<0.001
Number (%) ICU days spent with at least three antimicrobials	2721 (57.7%)	3570 (65.5%)	0.002

ICU: Intensive care unit. [a]This comparison was made with complete follow-up for 28 days (if patients were discharged from ICU, they were followed for antimicrobial use in all hospital admissions in Denmark). Pip/tazo: piperacillin/tazobactam. DDD: Defined Daily Dose administered within day 1-28. Parts of this table is also available in the primary publication on this material[13]. It is included in the present report since it is crucial for interpretation of the results.

Supplementary table 3: Cox proportional hazards models investigating predictors of mortality after ten days

	Unadjusted analysis		Multivariable analysis	
Variable	**Hazard ratio (95% CI)**	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Treatment arm ('High exposure vs. 'Standard exposure)	0.97 (0.72, 1.31)	0.86	0.93 (0.69, 1.26)	0.63
Hospital:				
1	Ref	0.11	Ref	0.37
2	0.63 (0.19, 2.05)		0.50 (0.15, 1.66)	
3	0.54 (0.17, 1.75)		0.49 (0.15, 1.63)	
4	0.86 (0.26, 2.81)		0.65 (0.19, 2.21)	
5	0.56 (0.16, 1.88)		0.45 (0.13, 1.56)	
6	0.71 (0.21, 2.37)		0.63 (0.18, 2.12)	
7	0.79 (0.23, 2.72)		0.66 (0.18, 2.40)	
8	0.43 (0.11, 1.53)		0.34 (0.09, 1.26)	
9	0.23 (0.05, 1.02)		0.27 (0.06, 1.26)	
Gender (Female vs. Male)	0.80 (0.59, 1.08)	0.14	0.77 (0.57, 1.05)	0.10
Age (≥65 years vs. <65 years)	1.96 (1.42, 2.69)	<0.0001	1.86 (1.34, 2.58)	<0.0001
APACHE II score (≥20 vs. <20)	1.77 (1.31, 2.39)	<0.0001	1.35 (0.98, 1.87)	0.07
Infection at baseline (Severe Sepsis or septic shock vs Milder or no infection)	1.31 (0.97, 1.76)	0.08	1.17 (0.84, 1.64)	0.35
Surgical patient (Yes vs. No)	0.78 (0.57, 1.06)	0.11	0.76 (0.55, 1.05)	0.09
Date recruited (01/01/08 to 02/06/09 vs. 09/01/06 to 31/12/07)	1.11 (0.81, 1.53)	0.50	1.18 (0.84, 1.67)	0.34
eGFR ever <30 mL/min/1.73 m^2 over the first ten days (Yes vs. No)	1.81 (1.34, 2.45)	<0.0001	1.47 (1.06, 2.04)	0.02

Diagram D1 Example of the site-specific interventional algorithm, site 'Aarhus'

The Procalcitonin And Survival Study (PASS) Intervention Algorithm, Site: Aarhus

IMPORTANT: All patients shall (at least) receive antimicrobial therapy covering "standard-of-care", i.e. if any existing guidelines or evidence for antimicrobial treatment indicate/ contra-indicate surgical and/or antibiotic treatment, then the patient should be treated according to this. Indicated treatment should never be left out because of a possibly low procalcitonin (PCT).

All (except for the above standing situations) patients in the "PCT intervention" group must have treatment according to the present guidelines, including interventions when procalcitonin is ≥1,0 ng/ml and "Alert"[a].

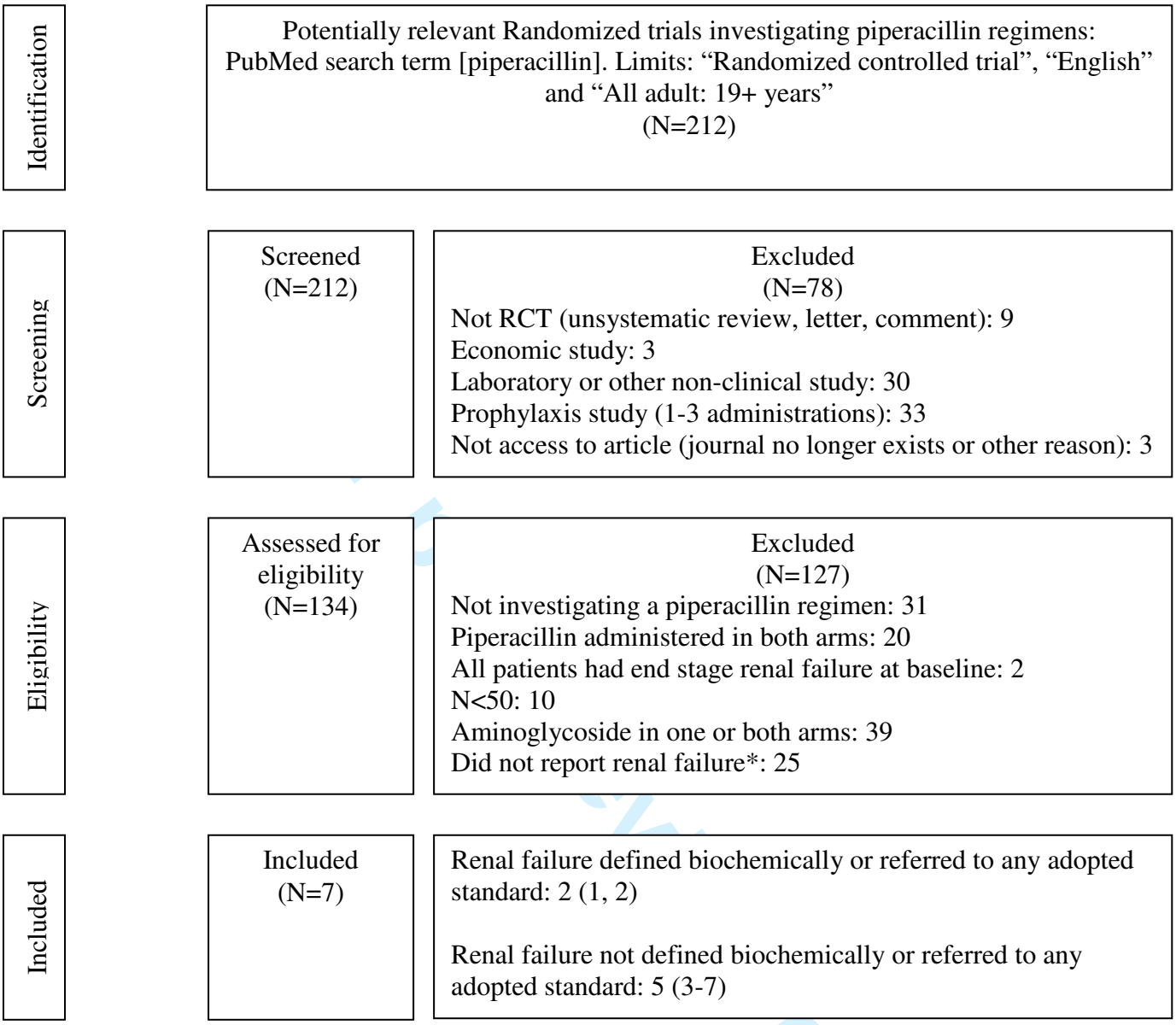
Patients are categorized daily according to the PASS intervention categories, on the basis on the present and the previous PCT measurement (displayed as "Alert" or "Non-Alert" in the website). In correspondence with every category, a PASS-intervention is displayed below. The treatment is, adjusted according to new and relevant microbiology that "explains" the clinical picture

CATEGORY 1	First PCT > 1,0 ng/ml, patient has not received antibiotics (≥1 DDD[b] within 72 h)
CATEGORY 2	A) First PCT ≥1,0 ng/ml, patient has received antibiotics (≥ DDD[b] within 72 h) or B) PCT "Alert" for 1 day after CAT 1,CAT 4 or CAT 5 has been started or C) PCT "Alert"** from "start-sample" till next morning
CATEGORY 3	A) First PCT ≥1,0 ng/ml, patient has received antibiotics (≥ DDD[b] within 72 h) and clinical suspicion of fungal infection or catheter related infection. or B) PCT "Alert" for 1 day after CAT 2 has been started
CATEGORY 4	A) Start PCT< 1,0 ng/ml or B) "Non-Alert" PCT, but ≥ 1,0 ng/ml. or C) PCT < 1,0 for 1-2 days
CATEGORY 5	PCT < 1,0 ng/ml for 3 or more days.

Action / Category	Diagnostics	Surgery	Antimicrobials[c]
CATEGORY 1	• Blood culture • Tracheal secretion • Urine culture • Culture from susp. source • Diagnostic imaging of susp. source	According to diagnostic imaging and clinical judgment	1. Cefuroxim 1500 mg x 3 i.v. or Ampicillin 1g x 4 / 2 g x 3 i.v. 2. Ciprofloxacin 400 mg x 2 i.v. 3. Consider: Metronidazol 500 mg x 2 i.v.
CATEGORY 2	• Blood culture • Tracheal secretion • Urine culture • Culture from susp. source • Diagnostic imaging of susp. source	According to diagnostic imaging and clinical judgment	1. Pip/Tazo[d] 4gx3 iv or Meropenem 1gx3 iv 2. Ciprofloxacin 400 mg x 2 i.v. 3. Metronidazol 500 mg x 2 i.v. 4. Consider fungal infection: Fluconazole i.v. and cath. inf: Vancomycin, dosage acc.to. Se-Vanco[e]
CATEGORY 3	• Blood culture • Tracheal secretion • Urine culture • Culture from susp. source • Diagnostic imaging of susp. source • Renewing oldest diagnostic imaging of susp. source	According to diagnostic imaging and clinical judgment	1. Pip/Tazo[d] 4gx3 iv or Meropenem 1gx3 iv 2. Ciprofloxacin 400 mg x 2 i.v. 3. Metronidazol 500 mg x 2 i.v. 4. Fluconazol 400 mg x 2 i.v. 5. Vancomycin, dosage acc.to. Se-Vanco[e]
CATEGORY 4	Nothing further	Standard-of-care approach	Continue present treatment
CATEGORY 5	Nothing further	Standard-of-care approach	Re-consider the indication for antibiotics (standard-of-care principle)

[a] 'Alert PCT' is defined as PCT-day1 ≥ PCT day 0 x 0.9. So a decrease in PCT from 11,2 ng/ ml to 10,5 ng/ ml is an "irrelevant decrease" and is defined as an "Alert" PCT. [b]DDD = Defined Daily Dosages). N.B.: The mentioned dosages are examples. Dosing regimen and frequency is prescribed according to the department guidelines (according to weight, kidney function, haemodialysis, Continuous dialysis etc.). [c]Antimicrobial spectrum covered can be broader than suggested (discretion of investigator). Administration of antimicrobials with a narrower spectrum on Alert-PCT days, should only take place when any antimicrobial treatment covering the suggested spectrum is contra-indicated and such a therapy should always be discussed and accepted by the coordinating centre. [d]Pip/Tazo: piperacillin/tazobactam. [e]Se-Vanco: serum-vancomycin measurements

Diagram D2:
Meta-analysis of randomized trials using piperacillin-containing regimens exploring renal failure



Results:

- In the initial identification phase, four ICU studies were found: They were excluded, since A) only a (non-defined) part of the patients received piperacillin(8), B) Both groups received piperacillin(9), C) one or both groups received aminoglycosides concomitantly(10, 11) .
- In the 7 (non-ICU) trials eventually included, 1592 episodes of therapy were observed.
- 21 cases of renal failure (not defined) occurred, corresponding to 1.3%.
- Hypothesizing, that the incidence of renal failure is 0.5% in non-piperacillin containing beta-lactam therapies, and aiming to find a risk increase to totally 1.5% (relative risk of 3.0), using conventional type I risk limit of 5% and a power of 80%, the sample size for such a trial investigating this should be approx. 3300 patients (non-ICU setting).
- In an ICU setting, the incidence of renal failure is often >20%. A trial of 1000 patients would be able to detect a risk increase to 28% (Relative risk:1.4) from e.g. piperacillin

*All articles were reviewed for this. Additionally, in adobe documents with the search option (those not scanned), a search was made in each pdf document with search terms: "renal", "kidney", "nephro", "creatinine" and "gfr". More than the noted 25 of the articles did not report renal failure, however, if they fulfilled one or more of the other exclusion criteria, they were excluded because of this.

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11. Brun-Buisson C, Sollet JP, Schweich H, et al. Treatment of ventilator-associated pneumonia with piperacillin-tazobactam/amikacin versus ceftazidime/amikacin: a multicenter, randomized controlled trial. VAP Study Group. *Clin Infect Dis*. 1998; 26: 346-54.

Table T1: Cox proportional hazards models investigating predictors of 'all cause' mortality on day 7-28

	Unadjusted analysis		Multivariable analysis	
Variable	**Hazard ratio (95% CI)**	**P-value**	**Hazard ratio (95% CI)**	**P-value**
Gender (Male vs. Female)	1.31 (0.99, 1.75)	0.06	1.30 (0.97, 1.73)	0.08
Age (≥65 years vs. <65 years)	1.90 (1.41, 2.55)	<0.0001	1.78 (1.31, 2.42)	<0.0001
APACHE II score (≥25 vs. <25)	1.67 (1.24, 2.25)	0.001	1.29 (0.94, 1.76)	0.12
Severe Sepsis/septic shock vs. Milder or no infection)	1.29 (0.97, 1.72)	0.08	1.31 (0.97, 1.76)	0.08
Surgical patient (Yes vs. No)	0.58 (0.41, 0.82)	0.002	0.54 (0.38, 0.77)	0.001
Cancer (Yes vs. No)	1.19 (0.79, 1.79)	0.41	NI	-
Charlson score (≥2 vs. <2)	1.69 (1.28, 2.24)	<0.0001	1.68 (1.22, 2.30)	0.001
eGFR <60 mL/min/1.73 m^2 on day 7 (Yes vs. No)	2.20 (1.66, 2.92)	<0.0001	2.29 (1.58, 3.34)	<0.0001

eGFR: estimated glomerular filtration rate; APACHE II: Acute Physiology And Chronic Health Evaluation II; NI: Not Included. Only patients who survived until day 7 were included in this analysis. Forward censoring was applied and variables with p<0.2 in the univariate analysis were entered into the multivariate model. If a creatinine measurement was not available on day 7, the last measured creatinine on day 1-6 was used. To not let hydration influence the eGFR estimations, baseline body weight was used for all daily eGFR estimations (so eGFR was a first degree function of measured creatinine for each patient). The analysis was stratified for baseline eGFR (<30 mL/min/1.73 m^2, 30-60 mL/min/1.73 m^2, >60 mL/min/1.73 m^2). In a sensitivity analysis, not stratifying for baseline eGFR did not alter the signal.

Protocol

A randomised, single-blinded, multicentre trial to investigate if clinical management guided by daily standardised Procalcitonin measurements can reduce the mortality in critically ill patients

The Procalcitonin and Survival Study (PASS)

Version of protocol: 3.1

Date: December 2006

Intensive Care Units from many University Hospitals all over Denmark will participate:

Sponsor: Scientific:
Copenhagen HIV Programme (CHIP) 044, Hvidovre University Hospital, Denmark
: Economic: Danish Research Council (Danish State) and other independent research foundations

Protocol co-ordinator

Jens-Ulrik Stæhr Jensen
H:S Hvidovre University Hospital
DK - 2650 Hvidovre
Denmark
Phone: +45 36 32 33 07
Fax: +45 36 47 33 40
E-mail: koordinator@pass-studiet.dk

INVESTIGATOR PROTOCOL AGREEMENT PAGE

THIS AGREEMENT IS EQUIVALENT TO A "SIGNED PROTOCOL"

The PASS Trial

Name and qualifications of investigator:

Name of Investigator: _____

Post held: _____

Clinical Centre: _____

I agree:

- to assume responsibility for the proper conduct of the PASS Trial at this site.
- to conduct the trial in compliance with this protocol, any future amendments, and with any other trial conduct procedures provided.
- not to implement any deviations from or changes to the protocol without agreement from the sponsor and prior review and written approval from the Independent Ethics Committee (IEC), except where necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the trial (where permitted by all applicable regulatory requirements).
- that I am thoroughly familiar with the appropriate use of the Procalcitonin test and the interpretation of the test results, as described in this protocol, and any other information provided by the manufacturer of the test and by the PASS Coordinating centre.
- that I am aware of, and will comply with, "Good Clinical Practice" (ICH-GCP Guideline (CPMP/ICH/135/95, Directive 2001/20/EC)) and all applicable regulatory requirements.
- to ensure that all persons assisting me with the trial are adequately informed about the Procalcitonin test and interpretation and of their trial-related duties and functions as described in the protocol.

_____ _____

Signature of investigator Date

One signed copy each to be held by the Investigator and PASS Co-ordinating centre.

15/10/2007

TABLE OF CONTENTS

A randomised, single blinded, multicentre trial to evaluate whether daily Procalcitonin measurements and immediate diagnostic and therapeutic response on abnormal values and day-to-day changes can reduce the mortality of critically ill patients in the Intensive Care Unit.

The Procalcitonin And Survival Study (PASS)

PROTOCOL SUMMARY

Inclusion:

Fulfilment of all of the following three criteria:

1 Male or female, aged ≥ 18 years of age.

2 Admitted to the participating intensive care units (ICU) at following hospitals: Hvidovre Hospital; Bispebjerg Hospital; Herlev Hospital; Glostrup Hospital; Gentofte Hospital; Hillerød Hospital; Roskilde Hospital; Århus University Hospital, Århus; Århus University Hospital, Skejby.

3 1) Ability to understand and provide written informed consent to participate in this trial,

or

2) Ability to understand and provide oral informed consent in presence of at least one impartial witness who should sign and personally date the consent form

or

3) The subjects legally acceptable representative can understand and provide written informed consent if the subject is not capable of this because of the present mental or physical condition of the subject.

Exclusion:

A subject will **NOT** be eligible for inclusion in this trial if any of the following criteria apply:

1. Subjects with known hyper-bilirubinaemia (>0.4 mg/ ml) or hypertriglyceridaemia (>10 g/l) since this can interfere with measurements. If subjects with unknown status on these points are included and have PCT measurements, the measuring-equipment will detect these conditions.

2. Subjects suffering from a blood disorder, where daily sampling of 7 ml of blood for maximally 28 days (210 ml distributed on 28 days) will be an inconvenience or a potential risk, which could compromise the safety of the subject.

3. Subjects who are pregnant or breast feeding

The *a priori* probability of surviving with the normal recommended diagnostics and treatment with the presently available means to detect infections and on the other hand the normal diagnostics and treatment together with daily Procalcitonin measurements and prompt clinical reaction should be equal.

Randomisation:

Two arms (1:1), n = 500 per arm:

Arm 1: Normal recommended diagnostics and treatment of infections in the intensive care unit (standard of care)

Arm 2: Normal recommended diagnostics and treatment of infections in the intensive care unit (standard of care) **and** Procalcitonin guided diagnostics and treatment of infection

Primary Trial Objective: To address whether daily Procalcitonin measurements and immediate diagnostic and therapeutic response on abnormal values and day-to-day changes can reduce the mortality of critically ill patients in the ICU.

Trial registration days: Intensive Care Unit admission day, running routine registration of examinations and blood tests, day of discharge or death, day 28 after admission, day 60, 90, 120 and 180 after discharge.

Data collection: The data collection will be simple and performed real time via fax.

1 TRIAL BACKGROUND AND RATIONALE

1.1 Background

1.1.1 *Sepsis and mortality in the Intensive Care Unit*

Sepsis remains a major cause of mortality in critically ill patients admitted to the Intensive Care Unit (ICU) [1-2]. All-cause mortality during ICU admission ranges from 12.1% in non-infected patients to 43.9% in infected patients[3]. Patients who are discharged to other departments and later to their own home or an institution for rehabilitation, continue to have a high mortality (additionally 10-20%) for 20-30 days after ICU discharge[4-7]. Different explanations for this have been proposed. Among the most important are:

1) During ICU admission it becomes clear that further treatment lacks perspective for the patient (often chronical organ diseases and cancer diseases) and the patient is therefore discharged to the relevant department when discharge from the ICU is possible.
2) After discharge from the ICU the physical condition of the patient deteriorates because of a severe disease with a dismal prognosis and it is decided together with the patient and relatives that the patient should not be admitted to the ICU again.
3) Critically ill patients often have an immunological incompetence and therefore these patients are susceptible to serious infections. Additionally these infections often have an atypical course and thereby a delayed diagnosis. This immunological incompetence prevails some time after discharge from the ICU why the patient remains susceptible to infections for this period of time. There is a grave risk that these serious infections with an atypical course can be diagnosed late in the course and cause an increased risk of mortality for critically ill patients.

1.1.2 *Procalcitonin and bacterial infections*

In 1993 Assicot et al. reported that a high level of serum-Procalcitonin (PCT) was closely related to bacterial infection and seemingly correlated to the severity of the infection[8]. This finding has since been ascertained in many studies demonstrating high levels (2.0 ng/ml-50.0 ng/ml (-1500 ng/ml)) of PCT in patients with systemic bacterial infection, while low levels have consistently been found in patients with localised bacterial infections and viral infections[9-16]. Others have shown low PCT levels (and seldom up till maximally 3.0 ng/ml) in non-infected patients following surgery, trauma and myocardial infarction[10, 17-21]. Sensitivity and specificity for sepsis when PCT levels are above 5.0 ng/ml have been estimated to 80-90 % and 85-100%, respectively, in the largest of these studies.
The PCT level starts decreasing within 24 h after surgery, trauma and myocardial infarction in non-infected patients in contrast to the C-reactive protein, which has a peak level 36-72 h after these events[10-17-21.]

Consequently, bacterial infection is suspected if PCT is increasing 24 h after surgery, trauma or myocardial infarction.

1.1.3 *Procalcitonin kinetics, biochemistry and cellular biology*

PCT is a 13 kDa, 116 amino acid polypeptide, initially described as a pro-hormone of Calcitonin, a

hormone in the calcium metabolism, which is produced in the medullary C-cells in the thyroid gland[22-24]. Recent studies have shown that the PCT variant, which is related to infection is produced in other tissues (liver, kidney, muscle, fat)[25-27]

Kinetic studies with healthy humans and baboons have shown a rapid release of PCT within 2-6 hours after injection of bacteria or bacterial endotoxin. This time to release is significantly shorter than that of C-reactive protein (8-24 h). The plasma half life of PCT is approximately 24 h. PCT measurements in healthy, uninfected volunteers has been shown very low levels (<0.05 ng/ml)[10,28-29].

1.1.4 *Procalcitonin-guided treatment and reduction in the use of antimicrobial agents*

A recent study has demonstrated a reduced use of antimicrobial agents in patients with lower respiratory tract symptoms, when the treatment was guided by the initial PCT level[30].

1.1.5 *Procalcitonin and risk of mortality*

We have shown that a PCT increase after reaching a level of 1.0 ng/ml is an independent predictor of mortality in critically ill patients. Patients who did not reach a PCT level above 1.0 ng/ml had an all cause mortality risk of 4.7% while admitted in the ICU, compared to an all cause mortality of 19.1% for the whole population of ICU patients. Patients who reached a PCT value above 1.0 ng/ml who had a decreasing PCT the next day had a mortality risk of 18.9%, but patients who had an increasing PCT level after reaching 1.0 ng/ml had a mortality risk of 32.7%. This increase in mortality risk was significant for the entire follow-up period of 90 days[31].

The mortality risk increased for every day the PCT increased. Taking in mind the close relation between PCT levels and bacterial infection, a large part of this mortality increase is (when PCT is increasing), to the best of the existing knowledge, attributable to uncontrolled bacterial infections. This is supported by the findings of the European Sepsis Group[3].

The rapid release of PCT to the blood stream (2-6 h), when infection is progressing, makes acute detection of ongoing serious infection possible, hereby potentially reducing mortality in critically ill patients if treatment is guided acutely by PCT measurements.

1.2 Rationale - summary

Sepsis and complications to sepsis are major causes of mortality in critically ill patients[1-2]. Rapid treatment of sepsis is of crucial importance for survival of patients. In the ICU, the infectious status of the patient is often difficult to assess because symptoms cannot be expressed (unconscious or sedated patients) and signs may present atypically because of immunologic incompetence and masking by the drugs given and thermo-influencing-therapy, i.e. dialysis. Biological and biochemical markers of inflammation (WBC, C-reactive protein) may often be influenced by other parameters than infection, such as: trauma, surgery, other types of inflammation such as rheumatoid diseases (C-reactive protein) and gluco-corticosteroid treatment (WBC), and may be unacceptably slowly released after progression of an infection[32-33]. At the same time, lack of a relevant antimicrobial therapy in an early course of infection may be fatal for the patient.

For these reasons, in the clinical setting, it is often necessary to initiate or adjust antimicrobial therapy on an unsure ground and the relevant therapy may in some situations be delayed for important hours or even days. Specific and rapid markers of bacterial infection have been sought for use in the ICU. Mortality in critically ill patients increases gravely when Procalcitonin levels increase from day to day[31]. Low PCT levels have been shown to effectively rule out sepsis[12].

However, no randomised controlled trials have been conducted to show if mortality in critically ill patients can be reduced by using a strategy of daily standardised Procalcitonin measurements as an early detector of serious bacterial infection. Therefore evidence is presently not sufficient to introduce daily consecutive Procalcitonin measurements to guide the diagnostic and therapeutic management of patients admitted to the ICU .

The rationale for this trial is to assess the ability of daily Procalcitonin measurements to reduce the mortality of critically ill patients.

1.3 Procalcitonin analysing methods

There are four commercially available analysing methods for measuring blood levels of Procalcitonin, one semi-quantitative and three quantitative. Two of these are described below, the oldest and most used test, *LUMITEST ® BRAHMS /BRAHMS PCT LIA,* and a newer fully automated test with a higher sensitivity, *KRYPTOR® PCT BRAHMS.* KRYPTOR® PCT BRAHMS will be used for all Procalcitonin analyses in this study[34].

1.3.1 LUMITEST ® BRAHMS /BRAHMS PCT LIA

The oldest and so far most used quantitative test is LUMITEST ® BRAHMS /BRAHMS PCT LIA. Analysis is made by a ”sandwich” luminiscens immuno-assay with an anti-catacalcin coated tube: Anti-**Catacalcin** binds catacalcin in the patient sample and is hereby immobilised (catacalcin could otherwise interfere with the analysis).
Anti-**Calcitonin** antibody is marked with a luminescent a*cridin*-derivative.
H_2O_2 and NaOH are added and these react with the *acridin*-derivative which leads to the formation of *acridon* and this process is accompanied by transmission of light. The quantity of this light is proportional to the Procalcitonin concentration in the sample.
We have found a coefficient of variation (CV) in the measuring interval between 0.1 ng/ml-1.0 ng/ml of 0.09-0.83 for this test. At PCT levels above 1.0 ng/ml, we found CV´s of 0.008-0.065 (range)[37].
The manufacturer claims a *functional assay sensitivity* (CV<0.2) of 0.3 ng/ml.

1.3.2 KRYPTOR® PCT BRAHMS

A new, and according to the manufacturer, more precise assay is the fully automated KRYPTOR® PCT BRAHMS. Procalcitonin is analysed using the analysing machine KRYPTOR® and fluids and utensils from the company BRAHMS diagnostica, Berlin. KRYPTOR® uses

TRACE technology (Time Resolved Amplified Cryptate Emission), which is a non-radiating transmission of energy. The transmission happens between two flourescent compounds: Europium Cryptate (donor) and XL665 (acceptor). While the antigen-antibody complex is formed, a signal is measured.

The functional assay sensitivity (CV< 0.2) is according to the manufacturer 0.06 ng/ml for the KRYPTOR ® test. In the relevant clinical interval (which has not quite been defined yet) the CV is 0.02-0.03 (product information).

- Studies concerning Procalcitonin have so far mainly been using LUMITEST ® BRAHMS /BRAHMS PCT LIA.

1.4 Rationale for a 24 h interval between blood sampling

Several studies have shown a half-life of Procalcitonin of 20-30 hours and Procalcitonin levels increase 2-6 h after bacterial products are presented in the blood stream [10,28-29, 35]. An important exception to this is patients suffering from severe uraemia, where the Procalcitonin half-life is prolonged, but it has been demonstrated, that Procalcitonin is removed by dialysis[35]. Studies concerning Procalcitonin and surgery have shown, that the Procalcitonin blood level is on a decreasing curve 24 h after major thoracic and abdominal surgery, except in infected patients[17-21]. In conclusion, a Procalcitonin level which is increasing 24 h after a therapy shift or after surgery suggests progression of infection.

1.5 Procalcitonin and immuno-compromised patients

Markers and mediators of inflammation and infection are often dependent on a functioning immune system, which is able to produce the substance measured, e.g. WBC, TNF, different interleukins[10,15,16, 36]. It has been established that Procalcitonin is not dependent on blood cells and their mediators, and Procalcitonin is mainly produced by tissues like liver, kidney, muscle and fat[25-28]. In concordance with this, studies investigating Procalcitonin in neutropenic patients have found results comparable to those for immuno-competent patients[36-41]. A few studies regarding neutropenic patients that compared PCT levels to positive blood cultures have found a low sensitivity of the test for bacteriemia, but these studies lack clear definitions of virulence of different micro-organisms (e.g. Coagulase negative staphylococci vs. Gram negative rods) in their study designs[40].

1.6 Studies on Procalcitonin biology and bacterial infection

1.6.1 In vitro and animal studies

In vitro studies have shown Procalcitonin to be an inducer of albumin synthesis in rat liver tissue measured on mRNA and protein synthesis. This was found to be opposite to TNFα and IL-6, these substances lowering albumin synthesis[42]. In a study of sepsis in baboons, low PCT was

found in non-infected subjects and high PCT in infected subjects, and PCT blood levels started increasing after 2 hours[10]. In another baboon model Procalcitonin incompetence was shown in an anhepatic subject[28].
In a study of burn wound and Pseudomonas aeruginosa septicaemia in rats, a high correlation between endotoxin levels and PCT in blood was found[43].

1.6.2 Human observational studies

Most of the present knowledge on Procalcitonin has been established by observational studies. Key-references are mentioned in paragraph 1.1 and 1.2

1.6.3 Clinical trials

Only few Randomized Controlled Trials regarding PCT-guided treatment have so far been published, one of special interest has used PCT-guided treatment (n=119+124)and has assessed the ability of this clinical strategy to reduce use of antimicrobial therapy in patients with suspected lower respiratory tract infection. A Relative Risk of 0.49 [95% CI 0.44-0.55] for antibiotic exposure was demonstrated, without any significant difference in culture growth from patient samples, quality of life, mortality, inflammatory parameters (temperature, C-reactive protein, WBC), number of days admitted and need for stay in intensive care unit. The study was designed to detect a 30 % difference with 95% stringency. However some of the mentioned endpoints do not occur in all patients, and in these cases (mortality, need for stay in ICU) it may be false to conclude, that there is no difference between groups within the chosen 30 % limit[30].
A very small study (n=12+13=25) has tried to investigate empiric prophylaxis with fluor-quinolone Ofloxacin in patients with abdominal aortic aneurism. However the sample size of this study does not justify any conclusions on this issue[44].

2 TRIAL OBJECTIVES AND ENDPOINTS

2.1 Trial Objectives

2.2 Primary Objectives

To address whether immediate diagnostic and therapeutic initiatives guided by abnormal high and increasing values of Procalcitonin measured daily can reduce the mortality of critically ill patients in the ICU.

2.3 Secondary Objectives

1. To determine mortality of ICU patients at discharge from the ICU, at day 60,90, 120 and 180.

2. To determine differences in prescription of antimicrobial therapy in the two arms.
3. To determine the frequency of patients with complications to infection in the two arms, defined as; sepsis, severe sepsis, septic shock, disseminated intravascular coagulation, multi-organ dysfunction syndrome (MODS), coma (Glasgow Coma Scale), hypotension, respiratory insufficiency (ventilator treatment need), liver insufficiency, acute uremia (three times increase in baseline creatinine).
4. APACHE II score
5. Accumulated PCT increases over time
6. To determine the number of diagnostic image procedures per day after enrolment in the trial in the two arms
7. To determine the number of non-routine microbiological samples taken per day after enrolment in the trial in the two arms
8. To determine the number of surgical procedures per day after enrolment in the trial in the two arms
9. To determine the time to the first change in antimicrobial chemotherapy after admittance to the ICU in the two arms

2.4 Trial Endpoint(s)

Primary:

Mortality at day 28 after admission to the ICU.

Secondary:

1. Mortality while admitted to the ICU, Mortality at day 60, 90 and 180 after admission to the ICU
2. Defined day doses of antimicrobial therapy in each arm
3. Occurrence of sepsis, severe sepsis, septic shock, DIC. Assessment of Glasgow Coma Scale, measurement of Blood Pressure (systolic blood pressure < 90), days with artificial ventilation, Factor 2-7-9 < 0.7, creatinine (increase factor 3 from baseline), MODS.
4. SOFA score daily (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale).

5. $AUC_{Procalcitonin}$ for the Procalcitonin-measuring group and for the control group.
6. Number of diagnostic images after admission to the ICU.
7. Number of non-routine microbiological sample taken after admittance to the ICU.
8. Number of surgical procedures during the trial
9. Time to the first change in antimicrobial chemotherapy after admittance to the ICU

3 INVESTIGATIONAL PLAN

3.1 Trial Design

3.1.1 Intervention

This is a randomised, single-blinded multicentre trial.

Approximately 1000 subjects admitted to an ICU in the participating University hospitals will be included. All patients included will receive the the standard recommended diagnostic and therapeutic procedures mandated at the particular ICU. Additionally, the patients will be randomised for:

1. No PCT guided diagnostics and treatment (i.e. the standard-of-care / **control arm**).

Or

2. Daily PCT measurements and protocol-specified additional diagnostic and/or therapeutic interventions guided by the PCT levels observed. High or increasing PCT levels will mandate such interventions (see section 3.3.1 for details of interventions)(the **PCT intervention arm**)

3.1.2 Randomisation

The randomisation is performed by the PASS study centre and is stratified according to site, age and initial Acute Physiology And Chronic Health Evaluation II (APACHE II) score. For patients randomised to the PCT intervention arm, daily PCT levels are communicated to the team responsible for the clinical management together with a recommendation of what interventions the investigator team is expected to initiate based on the PCT measurement. In

the control arm, blood samples for PCT will be analysed simultaneously with samples from the PCT intervention arm, but results of these PCT analyses will remain blinded for the investigators until the study has been completed. The PCT measurements will be conducted daily as long as the patient is admitted to the ICU, but maximally 28 days from time of enrolment in this study. While patients remain in the hospital, and after discharge from the ICU, samples will be collected for PCT determination but the samples will not be analysed real-time and hence the results will not be used to guide interventions outside the ICU, except if requested by the ICU investigator in conjunction with the discharge of the patient. Patients transferred from one ICU to another ICU, will remain in the trial provided that the receiving ICU also participates in this trial.

3.2 Trial Population

3.2.1 Inclusion Criteria

A subject will be eligible for inclusion in this trial only if all of the following criteria apply:

1 Male or female, aged ≥ 18 years of age.

2 Admitted to the participating intensive care units. Patients should be included within 24 h. If a patient has not been included at this time, this patient cannot be included in the present admittance.

3 Subjects should in the investigator's opinion be likely to be admitted to the ICU for more than 24 h. Subjects should not be likely (<10%) to die or be discharged in this period of time

4 Ability to understand and provide written informed consent to participate in this trial,

or

Ability to understand and provide oral informed consent in presence of at least one impartial witness who should sign and personally date the consent form

or

The subjects legally acceptable representative can understand and provide written informed consent if the subject is not capable of this because of the present mental or physical condition of the subject.

3.2.2 Exclusion Criteria

A subject will **NOT** be eligible for inclusion in this trial if any of the following criteria apply:

4. Subjects with known hyper-bilirubinaemia (>0.4 mg/ ml) or hypertriglyceridaemia (>10 g/l) since this can interfere with measurements. If subjects with unknown status on these points are included and have PCT measurements, the measuring-equipment will detect these conditions.
5. Subjects suffering from a blood disorder, where daily sampling of 7 ml of blood for maximally 28 days (210 ml distributed on 28 days) will be an inconvenience or a potential risk, which could compromise the safety of the subject.

3.3 Treatment During Trial

The aim of the PCT guided treatment is to reduce time to relevant treatment of a serious infection and thereby to reduce the mortality. All subjects will receive the standard-of-care evaluations and therapeutic interventions recommended in the ICU at which the patient is admitted to. Subjects in the PCT measurement group will additionally receive expanded diagnostics and treatment should the PCT levels be found to high and/or increasing (see section 3.3.1 for definitions).

Access to results of PCT measurements of any kind (semi-quantitative or quantitative) at any time in the study period is not allowed for patients randomised to the control arm.

The PASS study group in collaboration with the PASS Steering Committee, will issue guidelines for the composition of the interventions that a high or increasing PCT level would mandate. Some variation between sites is acceptable, whereas all patients within a given ICU should follow that ICU's guidelines. The guidelines will be updated when new information becomes available. In the guidelines, there may be several alternatives indicated for a given situation. The investigator is not mandated to follow the guidelines.

3.3.1 Procalcitonin levels and diagnostic and therapeutic consequenses

The situation mandating additional interventions in the the PCT intervention arm is based on the following criteria:

- PCT levels ≥ 1.00 ng/ml

and

- The PCT level increases one day to the next or has an irrelevant decrease of < 10%

The daily assessment of PCT guided interventions will be as follows:

- Subjects with PCT levels ≥ 1.00 ng/ml based on the first determination after enrolment into the study will follow the principles for interventions as detailed below.
- Subjects with PCT levels ≥ 1.00 ng/ml and with a day (n) to day (n+1) PCT increase or a decrease of < 10% (irrelevant decrease) will follow the principles for interventions as detailed below.
    - Microbiology: blood cultures, airway cultures, urine cultures and samples from any other suspected foci.
    - Considerations of whether to perform diagnostic imaging: one or more of the following: Chest X-ray, Ultra-sonic examination of suspected focus, Computerised Tomography of relevant areas, Magnetic Resonance imaging of relevant areas, other imaging techniques.
    - Surgical drainage of possible un-drained foci
    - Antimicrobial therapy expansion. Treatment will be guided by any relevant findings: microbial or diagnostic imaging, or other findings. If focus and micro organism of infection is not clear steps will be:

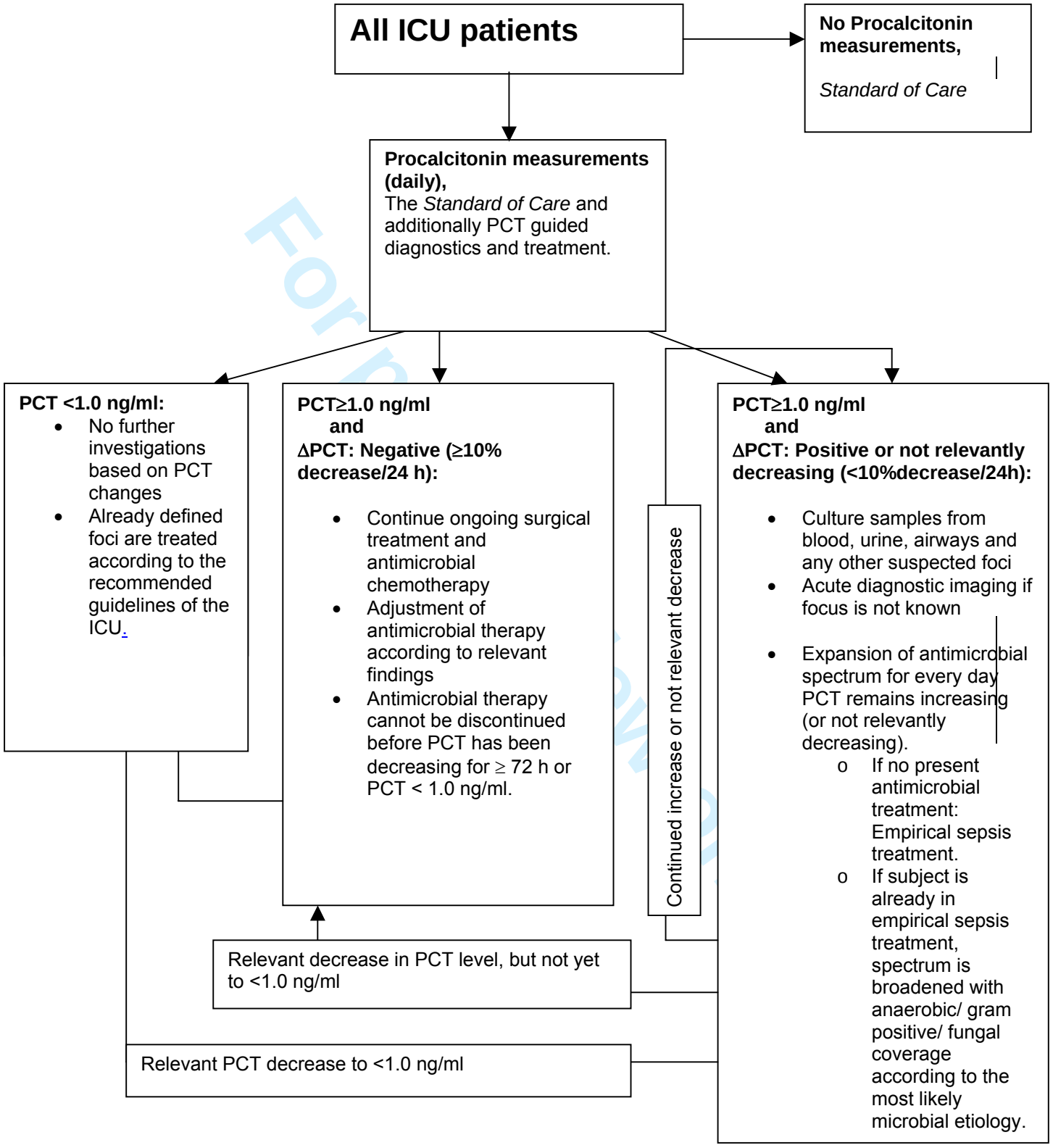
        1) Empirical sepsis treatment

        2) Empirical sepsis treatment with anaerobic and gram positive coverage

        3) Empirical sepsis treatment with anaerobic and gram positive coverage and/ or fungal treatment

- Subjects with PCT levels < 1.00 ng/ml will continue to receive standard-of-care
- Subjects with PCT levels ≥ 1.00 ng/ml and with a day-to-day PCT **decrease** of ≥ 10% will continue to receive standard-of-care.

Precise guidelines for this (antimicrobial) treatment will be made specifically for every ICU in concordance with the local choices regarding antimicrobial agents. For PCT guided diagnostics and treatment algorithm, see Diagram 1:



- **Antimicrobial treatment is NOT to be discontinued if PCT is increasing and > 1.0 ng/ml**
- **When treatment of infection is relevant, PCT normally decreases in less than 18 h. If PCT is still not decreasing at the next-coming measurement after a therapy shift, a new (expanded) strategy is to be instituted**

3.3.2 Change of PCT-guidance strategy during the trial

3.3.2.1 Randomised PCT-guided interventions

Subjects may **discontinue** the interventions initiated on the basis of PCT measurements only in case the benefit: risk ratio for these interventions is not acceptable to the treating physician. The specific concern will be collected.

3.3.2.2 The non-PCT guided interventions

The recommended interventions based on other information than PCT measurements should always be instituted and continued when relevant from a clinical judgement.

3.3.3 Antimicrobial Drugs and Dosages

All antimicrobial drugs prescribed on basis of an increasing PCT must be prescribed by the investigator or an intensive care physician, who has been sufficiently instructed in all aspects of the trial. The investigator must check for possible drug-drug interactions between any of the drugs prescribed guided by PCT changes and other agents that may be metabolised via the same enzyme systems or organs. To assist the investigator, information on this topic is included in the Manual of Operational Procedures. Also, the product label of each drug prescribed should be reviewed.

General principles that will be followed regarding antimicrobial therapy of sepsis are:

- Antimicrobial agents are prescribed, when possible, according to the resistance pattern of the causative microorganism.
- When the causative microorganism is not known, antimicrobial agents are prescribed according to knowledge of which microorganisms normally and possibly infect the suspected focus.
- When neither the microorganism nor the focus of infection is known, one or more broad spectrum antimicrobial agents are selected. If the effect is not sufficient, the spectrum of the used antimicrobial agents is additionally expanded, often with anaerobic active agents, gram positive active agents and antifungal agents. Conversely, if the effect is sufficient, the spectrum of used antimicrobial agents is narrowed according to knowledge of focus and causative microorganism.
- In empiric sepsis treatment, a combination of a ß-lactam/ Carbapenem + a fluor-quinolone is chosen if not contra indicated in the specific subject. This treatment can be

supplemented with nitroimidazoles, glycopeptides, oxazolidinones and azoles. More specific treatment regimes are initiated and guided by findings regarding the causative microorganism and/or focus of infection.

Dosages of antibiotics are decided according to the recommendations of the specific ICU.

The toxicity management guidelines detailed below refer to all components of the antimicrobial treatment used in the trial.

3.3.3.1 Overdose and Toxicity

Antimicrobial agents may be interrupted because of the development of adverse events (AEs, see section 6.1 for definitions) at the discretion of the investigator and according to the severity of the AE. The dose of all antimicrobial drugs may be reduced, interrupted or reintroduced according to standard practice at the time, and depending on the severity of the AE.

Subjects who require a dose modification should be re-evaluated on a daily basis.

The investigator is responsible for taking appropriate precautions to ensure that the risk of developing toxicity is minimised, that the subject is monitored for the development of toxicity, and if such toxicities do occur, take appropriate action to minimise their effects.

4 MEASUREMENTS AND EVALUATION

4.1 Time and Events Schedule

A flow chart showing the timing of trial procedures (Clinical and Laboratory) is shown in Table 1.

An initial pre-entry (screening) assessment for eligibility will be performed as soon as possible after the patient is admitted to the ICU. The patient should be randomised no later than 24 hours after the time of admission. Evaluations will then be carried out at entry (Day 1), and thereafter daily as long as the patients remains in the ICU. After discharge, the course of disease is collected in less detail and the survival status determined day 28, 60, 90 and 180 after enrolment in the trial.

4.1.1 Pre-entry Evaluations

The site must obtain subject consent in the form of a written informed consent form prior to the initiation of **any** pre-entry procedures as outlined in this protocol. The consent form must be approved by the IEC of each participating site.

The pre-entry evaluation will be conducted the first day of the trial by an investigator in the ICU and will include an evaluation of whether the patient fulfils the requirements for enrolment in this trial (see section 3.2.2 and 3.2.3.

Subjects who fail to meet the entry criteria may not be re-screened for this protocol until 28 days after the failed pre-entry evaluation. Hence, enrolment of such patients will require that the patient is re-admitted to the ICU after at least 7 days outside of the ICU after the time of the first screening.

4.1.2 Baseline (Day 1) Evaluations

The following evaluations should be performed at baseline (Day 1):

Note: For this trial, Baseline (Day 1) is defined as the day on which the subject has his/her first blood sample for PCT measurement. The following data are to be collected on day 1:

- Demography including date of birth, weight, height, and indication for admittance to the ICU
- Infections found in the subject in this hospital admission prior to admittance to the ICU.
- Present infection focus/ etiologic microorganism

- APACHE II score (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale)

- Current medical conditions

- Pre-admittance daily function and health state:

Professional career:	1) Student, 2) Part time work, 3) Full time work, 4) Early retirement, 5) Retired
Health:	1) Congenital handicapped, 2) Acquired handicap, 3) Chronic disabling disease, 4) Chronic non-disabling disease, 5) Healthy
Self-supportance:	1) Lives in nursing home, 2) Lives in a flat connected to a nursing home, 3) Own home with external help ≥ once / day, 4) Own home with external help < once daily, 5) Own home, no help required
Hospital need:	1) ≥ 3 months admitted to a hospital/ last year, 2) 1-3 months admitted to a hospital/ last year 3) 1-30 days admitted/ last year, 4) No admissions, ambulatory visits ≥ 6/ last year, 5) No admissions, ambulatory visits 1-5/ last year, 6) No admissions, No ambulatory visits/ last year

- Adverse events/ other complications to treatment given in this hospital admission (ongoing clinical conditions at Day 1 shall be recorded in the “Adverse Event and Medical Condition Form” of the CRF at this time, regardless of the fact that such conditions may not subsequently be found to fulfil the definitions for an adverse event (see section 6.1))

- Haematology: *haemoglobin, platelet count (WBC count mentioned as part of APACHE II)*

- Clinical chemistry: Albumin, Bilirubin, Factor 2-7-9, Alanin Amino Transferase (ALAT)/ Aspartate Amino Transferase (ASAT), Alcaline Phosphatase, Creatinine, Carbamide, Na^+, K^+, Phosphate, Ca^{2+}, C-reactive protein (some are also mentioned as part of APACHE II).

- Baseline PCT

The daily PCT determination is done real-time at the Department of Clinical Biochemical Department, Hvidovre Hospital, using the EC-approved measuring instruments and reagents. For each subject, the same methodology should be used throughout the trial period. The KRYPTOR® PCT BRAHMS sensitive assay is the accepted standard assay. Other licensed assays may be used instead if judged by the PASS steering committee to have a comparable performance compared to the indicated assay.

4.2 On Trial Evaluations

On trial assessments will be completed at the following time-points unless otherwise specified:

While admitted to the ICU, the following information will be registered unless specified otherwise:

Daily while patient is admitted to the ICU:

- Clinical signs of new (nosocomial) infections
- Microbiological or radiological evidence of new (nosocomial) infection
- Defined Day Doses of antimicrobial chemotherapy
- APACHE II score (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale)
- Occurrence of sepsis, severe sepsis, septic shock, DIC. Assessment of Glasgow Coma Scale, measurement of Blood Pressure (systolic blood pressure < 90), days with artificial ventilation, Factor 2-7-9 < 0.7, creatinine (increase factor 3 from baseline), MODS.
- Adverse events/ other complications to treatment given in the ICU (ongoing clinical conditions at Day 1 shall be recorded in the “Adverse Event and Medical Condition Form” of the CRF at this time, regardless of the fact that such conditions may not subsequently be found to fulfil the definitions for an adverse event (see section 6.1))
- Haematology: haemoglobin, platelet count WBC (WBC count also mentioned as part of APACHE II)
- Clinical chemistry: Albumin, Bilirubin, Factor 2-7-9, Alanin Amino Transferase (ALAT)/ Aspartate Amino Transferase (ASAT), Alcaline Phosphatase, Creatinine, Carbamide, Na^+, K^+, Phosphate, Ca^{2+}, C-reactive protein (some are also mentioned as part of APACHE II).

- Blood sample for PCT determination
- Diagnostic imaging procedures performed
- Non-routine microbiological sample taken
- Surgical procedures performed
- Change in antimicrobial chemotherapy

At the day of discharge from ICU or day of death or later:

- Mortality and time of death, and the cause hereof
- $AUC_{Procalcitonin}$ (at discharge from the ICU) (will remain blinded in the control arm)
- Discharge and post-discharge daily function and health state (obtained on day 30 and 180):

Professional career:	1) Student, 2) Part time work, 3) Full time work, 4) Early retirement, 5) Retired
Health:	1) Congenital handicapped, 2) Acquired handicap, 3) Chronic disabling disease, 4) Chronic non-disabling disease, 5) Healthy
Self-supportance:	1) Lives in nursing home, 2) Lives in a flat connected to a nursing home, 3) Own home with external help ≥ once / day, 4) Own home with external help < once daily, 5) Own home, no help required.
Hospital need:	1) ≥ 3 months admitted to a hospital/ last year, 2) 1-3 months admitted to a hospital/ last year 3) 1-30 days admitted/ last year, 4) No admissions, ambulatory visits ≥ 6/ last year, 5) No admissions, ambulatory visits 1-5/ last year, 6) No admissions, No ambulatory visits/ last year

After discharge from ICU while patient is still admitted to hospital

- Clinical signs of new (nosocomial) infections

- Microbiological or radiological evidence of new (nosocomial) infection
- Defined Day Doses of antimicrobial chemotherapy
- Current medical conditions (including acute organ failures)
- Diagnostic imaging procedures performed
- Surgical procedures performed
- Blood sample for PCT determination – done daily

4.3 Trial drugs

Drugs prescribed on basis of PCT levels and changes belong to following categories: Antibacterial chemotherapeutics and Antifungal chemotherapeutics. Drugs from these categories will also be prescribed for the control group (and in patients not included in the trial), when indicated from other findings than level/change of PCT. An exhaustive list of drugs, used in the participating ICU´s (and thereby also in the trial subjects and controls) is given in appendix

4.3.1 Dosing Details

The following details on dosing of all prescribed antimicrobials during the study period must be recorded in the “Medication form” in the CRF.

- Date of initial therapy
- Dose at each dosing change, together with reason for change
- Date of last dose of each agent
- Reason for discontinuation
- Date of resumption of therapy

4.3.2 Collection of Blood Samples for Daily Analysis

Plasma from the PCT group and the control group will be collected early each morning (01.00 a.m.-06.00 a.m.) and will be transported to the Department of Clinical Microbiology Hvidovre Hospital, DK-2650 Hvidovre (or other laboratories, that can provide a PCT analysis real-time and with an analysing method which is approved by the PASS coordinating centre) and analysed immediately hereafter. The results from this analysis will be communicated via a

webbased cryptized licensed answering system every day to the Intensive Care Units for patients randomised to the PCT intervention arm or concealed for patients randomised to the control arm. Remaining material for the blood samples will hereafter be frozen for later analysis of other biochemical, biological and genetic markers (-80°C). Once the trial has been completed, the coupling of these samples to person-identifiers will be broken, and hence subsequent analyses done without any possibility to connect the results to individual persons involved in the trial. For detailed instructions regarding the collection, labelling, processing and transport of samples, see the Manual of Operational Procedures.

It is the responsibility of the investigator (to be assisted by the courier service and PASS coordinating office) to ensure that all trial samples for transport are appropriately handled, packed and transported.

4.3.3 Genetic markers (PASS-sub-study)

The PASS-sub-study has three aims: 1. quality assessment of the procalcitonin analyzes used in the PASS-Study, 2. to investigate the relation between levels of procalcitonin and other biomarkers and 3. to investigate if genetic markers can be used to gain an early knowledge of the course of critical illness.

To investigate this, we will use the remaining material from the blood samples collected for the PASS-Study. Blood plasma and DNA material will be frozen at minus 80 degrees Celcius. The PASS-Sub-study, therefore, will not mean any inconvenience for the study subjects and no additional blood sampling. This material will be kept in anonymous form for 5 years after the closure of the PASS-Study. Known hereditary diseases will not be examined.

Regarding 1.: In a randomly assigned set of blood samples, and additionally in samples that have shown extreme PCT values a double determination will be performed to assess the inter-assay variability.

Regarding 2.: Other biomarkers as interleukin-6 and soluble TNF-α receptor have been, and are still under assessment as predictive markers at sepsis and in other infectious diseases. In plasma, these and other markers will be analyzed after the closure of the PASS-Study to assess the value of these markers compared to PCT, also as prognostic markers.

Regarding 3.: Genetic polymorphisms (e.g. mannan-binding lectins, interleukins, complement, immunglobulin receptor, Toll-like receptor 1-9, and Factor V Leiden) are related to the prognosis at sepsis and can, to some degree, identify patient groups with a high risk of a fatal course of

the disease. An increasing number of international studies have during the latest years investigated the relation between the genetic disposition of patients and the course of infectious diseases, but often, these studies have been small and without sufficient statistical power to conclude on these issues.
The statistical power in investigating the relation between genetic polymorphisms and mortality in sepsis depends on the frequency of a certain allele, the mortality in the study population and the size of the population.

Directly applied on the study population of the PASS-Study with 1000 cases of sepsis (mortality ~25%) it will result in a 80 % statistical power to show a 2-fold increase in mortality for an allele that is found in 3% of the population. For alleles that are more frequent, we will be able to show less than a 2-fold increase in mortality. As an example of this, the homozygote forms of TNF-α, IL-1β, and PAI-1 have a frequency of 5, 7, and 14%, respectively. Heterozygote forms of TLR4 and factor V Leiden have a frequency of 9 and 7%.

5 DATA ANALYSIS METHODS

5.1 Sample Size Determination

The trial will randomise (1:1) 1,000 subjects into two treatment arms:

1: Control arm

2: The PCT guided intervention arm

With a sample size of 500 per group and an assumed mortality rate of 25% in the control group and 17.5 % in the PCT group there will be 80% probability that a negative result (Confirming the Null Hypothesis) is true. At the same time there will be < 5% probability of falsely declaring the alternative hypothesis correct. [Power 80%, stringency 5%]. Sample Size calculations via Dept. of Statistics, UCLA, California, USA.

5.2 General Considerations

5.2.1 Analysis Populations

The primary population for analyses of the efficacy and safety data will be the intention to treat population, including all randomised subjects who have at least one blood sample made for PCT measurements.

Response to PCT guided diagnostic and therapeutic interventions will also be investigated descriptively by summary statistics for various sub-groups, e.g. gender, other demographic variables, Baseline APACHE II score, and pre-admittance health assessment.

5.2.2 Interim Analysis

Safety and efficacy data will be reviewed when 250, 500 and 750 subjects have completed the trial period (until discharge from the hospital or death, maximally 28 days), or at least every 6 th month, and assessments will be made by an independent Data and Safety Monitoring Board (DSMB). A cut-off date will be specified at this point and all treatment failure and adverse event data before this date will be used.

The Peto method of repeated significance testing will be used to test for treatment difference and a p-value of 0.001 will be used as the significance level at the interim analysis, giving a significance level of 0.05 for the final analysis once all patients have completed the trial.

Stopping the trial will not be based purely on a statistical decision but also on the recommendation of the DSMB.

5.2.3 Other Issues

All subjects will remain in the trial and be followed-up until day 180.

5.3 Efficacy

5.3.1 Primary Efficacy Endpoint

The primary efficacy analysis will be the comparison of the two treatment groups with respect to the incidence of mortality within 28 days after enrolment in the trial. Mortality is defined as all-cause mortality. Subjects not followed for the entire duration of the trial (i.e. lost to follow-up) will be counted as survivors. Very few patients will be lost to follow up for the primary endpoint, because of the Danish Central Person Register (CPR), where all deaths in Denmark are registered. Only subjects who permanently move their address to another country within 30 days after ICU admission can be lost to follow-up. The stratified log-rank test and Kaplan Meier estimates will be used.

5.3.2 Secondary Efficacy Endpoint(s)

5.3.2.1 Other mortality assessments

The proportion of subjects, who survive to different points of time (at discharge, after 60, 90 and 180 days, counting after ICU admission). The log rank test and Kaplan-Meier estimates will be used. Differences in proportions of survivors will be assessed using the Mantel-Haenzel Chi Square test and Wilcoxon test. Subjects with missing mortality data will be classified as survivors.

5.3.2.2 Other parameters than mortality

- Defined day doses of antimicrobial therapy in each arm
- Occurrence of sepsis, severe sepsis, septic shock, DIC. Assessment of Glasgow Coma Scale, measurement of Blood Pressure (systolic blood pressure < 90), days with artificial ventilation, Factor 2-7-9 < 0.7, creatinine (increase factor 3 from baseline), MODS.
- SOFA score daily (Temperature, Mean Arterial Pressure, Heart Rate, Respiratory Rate, FIO_2, HCO_3^-, pH (arterial), Se- Na^+, K^+, Creatinine, Haematocrite, White Blood Count+ differential count, Glasgow Coma Scale).
- $AUC_{Procalcitonin}$ for the Procalcitonin-measuring group and for the control group.
- Number of diagnostic images after admission to the ICU.
- Number of non-routine microbiological sample taken after admittance to the ICU.
- Number of surgical procedures during the trial
- Time to the first change in antimicrobial chemotherapy after admittance to the ICU
- Occurrence of new clinically, microbiologically or radiologically diagnosed infections while admitted to the ICU
- Discharge and post-discharge daily function and health state

For endpoints that have normally distributed numbers, t-test will be used in assessment of statistical significance. If not normally distributed, Mantel-Haenzel Chi Square test and the Wilcoxon test, will be used.

Exploratory analysis of adjustments for possible confounders present at baseline for the analysis presented above will be performed using Cox proportional hazards and Logistic regression modelling (as appropriate).

5.3.3 Combined evaluation of mortality / occurrence of serious bacterial infection while admitted to the ICU

The proportion of patients who die during the trial period or who experience occurrence of a serious bacterial infection (sepsis, severe sepsis, septic shock, Disseminated Intravascular Coagulation (DIC) or Multi Organ Dysfunction Syndrome (MODS) (which ever came first) as a function of time since trial initiation. In this analysis, patients discontinuing the randomised treatment for other reasons before having failed in this analysis will be censored from the time of discontinuation.

5.4 Safety

Adverse events will be tabulated by treatment group, maximum intensity, attributability to various antimicrobial agents and by seriousness. Treatment related adverse events that lead the subject to prematurely discontinue one or more of the originally prescribed antimicrobial agents will also be summarised.

Clinical chemistry and haematology results will be presented by summary statistics and quartile plots of measured results. Change from baseline for these results will also be presented. Baseline is defined as the laboratory data collected at Day 1 (before the first blood sample for PCT analysis). Subjects must have both a baseline and an “on treatment” measurement to be included in the change from baseline analysis.

Treatment emergent toxicity grades will be presented for each graded laboratory parameter by treatment group. A graded toxicity is considered treatment emergent if it develops or increases in intensity, post Day 1. Treatments will include established and approved antimicrobial treatments, which are already used daily in the participating ICU´s.

Concurrent medications and blood products will be summarised by randomised treatment group.

6 ADVERSE EVENTS (AE) AND SERIOUS ADVERSE EVENTS (SAE)

As mentioned other places in this protocol, the direct inconvenience for subjects in this study is sampling of 7 ml of whole blood daily in the same session as the routine blood samples are made, every morning. Therefore it is reasonable to expect that AE´s and SAE´s as a direct consequence of this blood sampling will not occur. Indirect AE´s as a consequence of potential overly treatment are likewise not likely to occur according to the available literature on the issue, especially because the most striking result of the previously published RCT´s is a reduction of antibiotic exposure in the PCT-guided group.
All interventions, that are performed in this study are well-known, thoroughly tested and accepted treatments, so it does not seem reasonable to apply the same procedures for this study regarding AE´s as e.g. a study where a new drug is to be assessed for safety (or effect)

Investigators will, however, have the opportunity to report events, that they fing unexpected in the Case Report Form. In this part of the CRF, it is possible to classify unexpected events in groups of "relatedness" to the antimicrobial treatment as "no relation", "unlikely relation", "possibly related", "probably related" or "definitely related.

Serious unexpected events or unexpected events

Serious inexpected events and unexpected events, that can be related to the antimicrobial treatment will in both treatment groups be reported to the Danish Medicines Agency "Lægemiddelstyrelsen" according to the Danish legislation on this point

The primary and the secondary endpoints that are registered daily in the case report form are all *adverse events or serious adverse events, i.e. death, complications to sepsis, increased antibiotic exposition and prolonged hospital stay. These are registered routinely and daily in the part of the CRF dealing with effects of the treatments. All patients are at inclusion in the study threatened by potentially lethal illnesses.*

7 TRIAL ADMINISTRATION

7.1 Data Collection

Case Report Forms (CRF) will be provided for each subject by the PASS coordinating centre. All data on the CRFs must be entered legibly in black ink or typed, in Danish or English. Amendments and errors on the CRFs should not be erased, covered with correction fluid or completely crossed-out; rather, a single line should be drawn through the error and the correction initialled and dated by the investigator, authorised colleague or co-worker. An explanatory note for the change should also be written on the CRF. Any requested information which is not obtained or unanswerable should be identified by entering 'ND' (not done). An explanation must be documented for any missing data. CRFs must be completed regularly and should never bear the participant's name. Participants will be identified by initials, date of birth and subject trial number only.

The investigator (or a person appointed by the investigator) must sign and date a declaration on the CRF attesting to his/her responsibility for the quality of all data recorded and that the data represents a complete and accurate record of each subject's participation in the trial.

Details and procedures for the completion of the CRFs are specified in the Manual of Operational Procedures.

All trial CRFs will be plain paper copies – the original being the investigators copy. After completion of each page of the CRF, the investigator will send it by fax to the PASS coordinating centre. Pages will be reviewed and clarified in accordance with the protocol specific Review and Validation Manual. The data will be double entered (punched and verified) by separate data entry specialists to produce data files.

Identical validation checks will be performed on each database. Data failing any check will be flagged for output on a Data Clarification Report (DCR) and sent to the relevant investigator for resolution. In such cases the investigator is requested to sign and date any explanation or correction. On return, the database will be updated appropriately and the original DCR stored with the original CRF.

The database(s) will be subject to agreed Quality Control (QC) checks before authorisation. The data will be subsequently analysed according to the methods outlined in Section 5.

7.2 Regulatory and Ethical Considerations

7.2.1 Regulatory Authority Approval

The co-ordinator (in collaboration with the PASS coordinating centre) will obtain approval from the appropriate regulatory agency prior to initiating the trial at a site.

This trial will be conducted in accordance with ICH-GCP and all applicable regulations, including, where applicable, the Declaration of Helsinki, June 1964, as modified by 52nd WMA General Assembly, Edinburgh, Scotland, October 2000 (see Appendix 1).

7.2.2 Ethics Approval

It is the investigator's responsibility to ensure that this protocol is reviewed and approved by the appropriate local Independent Ethics Committee (IEC). The IEC must also review and approve the site's informed consent form (ICF) and any other written information provided to the subject prior to any enrolment of subjects, and any advertisement that will be used for subject recruitment. The co-ordinator and/or the investigator must forward to the PASS coordinating centre copies of the IEC approval and the approved informed consent materials, which must be received by the PASS coordinating centre prior to the start of the trial.

If, during the trial, it is necessary to amend either the protocol or the informed consent form, the co-ordinator and/or investigator will be responsible for ensuring the IEC reviews and approves these amended documents. IEC approval of the amended ICF must be obtained before new subjects consent to take part in the trial using this version of the form. Copies of the IEC approval of the amended ICF and the approved amended ICF must be forwarded to the PASS coordinating centre as soon as available.

7.2.3 Subject Informed Consent

The investigator or his/her designee will inform the subject of all aspects pertaining to the subject's participation in the trial.

The process for obtaining subject informed consent will be in accordance with all applicable regulatory requirements. The investigator or his/her designee and the subject/ witness of an oral informed consent/ subjects legally acceptable representative must both sign and date the ICF before the subject can participate in the trial. Following types of informed consent can be accepted because of the nature of the ICU setting and the physical and/ or mental state of the subjects.

1)Ability to understand and provide written informed consent to participate in this trial,

or

2)Ability to understand and provide oral informed consent in presence of at least one impartial witness who should sign and personally date the consent form

or

3)The subjects legally acceptable representative can understand and provide written informed consent if the subject is not capable of this because of the present mental or physical condition of the subject.

The subject will receive a copy of the signed and dated form and the original will be retained in the site trial records. The decision regarding subject participation in the trial, that is made by the subject, is entirely voluntary. The investigator or his/her designee must emphasize to the subject that consent regarding trial participation may be withdrawn at any time without penalty or loss of benefits to which the subject is otherwise entitled.

If the ICF is amended during the trial, the investigator must follow all applicable regulatory requirements pertaining to approval of the amended ICF by the IEC and use of the amended form (including for ongoing subjects).

7.3 Trial Monitoring

In accordance with applicable regulations, good clinical practice (GCP), monitors will periodically contact the site, including conducting on-site visits. The extent, nature and frequency of on-site visits will be based on enrolment rate, the quality of the documents provided by the site, consistency of follow-up of the patients according to this protocol.

During these contacts, the monitor will:

- check and assess the progress of the trial

- review trial data collected
- conduct Source Document Verification
- identify any issues and address their resolution

This will be done in order to verify that the:

- data are authentic, accurate, and complete
- safety and rights of subjects are being protected
- trial is conducted in accordance with the currently approved protocol (and any amendments), GCP, and all applicable regulatory requirements

The investigator agrees to allow the monitor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the monitor to discuss findings and any relevant issues.

In addition to contacts during the trial, the monitor will also contact the site prior to the start of the trial to discuss the protocol and data collection procedures with site personnel.

At trial closure, monitors will also conduct all activities as indicated in Section 7.5, Trial and Site Closure.

7.4 Quality Assurance

At its discretion, the PASS coordinating centre may conduct a quality assurance audit of this trial. If such an audit occurs, the investigator agrees to allow the auditor direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor to discuss findings and any relevant issues. A guideline for audit is available at the PASS coordinating centre.

In addition, regulatory agencies may conduct a regulatory inspection of this trial. If such an inspection occurs, the investigator agrees to allow the inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the inspector to discuss findings and any relevant issues.

7.5 Trial and Site Closure

Upon completion of the trial, the following activities, when applicable, must be conducted by the monitor in conjunction with the investigator, as appropriate:

- return of all trial data to the PASS coordinating centre

- data clarifications and/or resolutions
- review of site trial records for completeness
- shipment of stored samples to assay laboratory

In addition, the steering committee reserves the right to temporarily suspend or prematurely discontinue this trial either at a single site or at all sites at any time and for any reason. If such action is taken, selected members of the PASS steering committee and/or the PASS coordinating centre will discuss this with the Investigator (including the reasons for taking such action) at that time. The PASS coordinating centre will promptly inform all other investigators conducting the trial if the trial is suspended or terminated for safety reasons. The investigators will inform their local/regional/national regulatory authorities (as appropriate) of the suspension or termination of the trial and the reason(s) for the action. If required by applicable regulations, the investigator must inform the IEC promptly and provide the reason for the suspension or termination.

If the trial is prematurely discontinued, all trial data must be returned to the PASS coordinating centre.

7.6 Records Retention

In accordance with applicable regulatory requirements, following closure of the trial, the investigator will maintain a copy of all site trial records in a safe and secure location. The PASS coordinating centre will inform the investigator of the time period for retaining these records in order to comply with applicable regulatory requirements.

7.7 Information Disclosure and Inventions

7.7.1 Confidentiality

The investigator and other trial site personnel will keep confidential any information provided by the co-ordinating centre (including this protocol) related to this trial and all data and records generated in the course of conducting the trial, and will not use the information, data, or records for any purpose other than conducting the trial. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the investigator or trial site personnel; (2) information which it is necessary to disclose in confidence to an IEC solely for the evaluation of the trial; or (3) information which it is necessary to disclose in order to provide appropriate medical care to a trial subject.

7.7.2 Publication

The findings from this trial is intended to be published in peer-reviewed journals. The steering committee decides whether abstracts are to be submitted to conferences, and how the results are distributed if more than one manuscript is to be drafted.

Authorship: The trial group as a whole will appear in an appendix in all published manuscripts. Co-authors are selected after a fair evaluation of primarily number of patients entered in to the trial and the level of involvement in the drafting of the manuscript. Providing that several manuscripts are to be drafted, a fair rotation among the participating clinical sites of co-authorship slots will be done taking in to consideration the number of patients enrolled.

7.8 Indemnification and Compensation for Injury

The insurance that covers liability in relation to patient care in Denmark, *Patientforsikringen* will cover all liability aspects of the conduct of this trial[45-46].

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Table 1: Clinical and laboratory Evaluations

Evaluation	Day (screening & baseline)		Day (counting after admission to ICU) (follow-up)				
	1	Day=Dis-charge/ death	28	30	60	90	180
Informed Consent	X						
Entry Criteria	X						
Demography	X						
APACHE II	X	X					
Infections during this hospital admission	X						
Current medical conditions	X	X					
State of daily function and health	X			X			X
Mortality		(X)	X		X	X	X
Baseline PCT	X						
$AUC_{procalcitonin}$		X					
Concurrent Medications[a]	X	X		X	X	X	X
Haematology	X	X					
Clinical chemistry	X	X					
Adverse events	X[a]	X					
Serious Adverse Events	X[a]	X		X	X	X	X

a Adverse events and serious adverse events are registered daily

9. APPENDICES

Appendix 1

Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly
Helsinki, Finland, June 1964
and amended by the
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
and the
52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.
9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.
19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
20. The subjects must be volunteers and informed participants in the research project.
21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.
23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.
24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain

informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Appendix 2: Abbreviations

AE	**Adverse Event (AE)**
ALAT	**Alanine Aminotransferase (SGOT)**
APACHE II	**Acute Physiology And Chronic Health Evaluation II**
ASAT	**Aspartate Aminotransferase (SGPT)**
CDC	**Centers for Disease Control**
CRF	**Case Report Form**
DDD	**Defined Day Doses**
DIC	**Disseminated Intravascular Coagulation**
DSMB	**Data Safety Monitoring Board**
ICU	**Intensive Care Unit**
IEC	**Independent Ethics Committee**
IL-6	**Interleukin 6**
MODS	**Multi Organ Dysfunction Syndrome**
PASS	**Procalcitonin and Surivival Study**
PCT	**Procalcitonin**
SAE	**Serious Adverse Event**
TNFα	**Tumor Necrosis Factor α**
WBC	**White Blood cell Count**

Appendix 3: Table of conversion factors for laboratory units

TEST	CONVENTIONAL		SI	
	Unit	Factor	Unit	Factor
Haemoglobin	g/dl	0,6206	mmol/l	1,61
Platelets	Thou/mm^3	0,001	[a]x10^9/l	1000
Hyponatraemia (↓ Sodium)	mEq/l	1,0	mmol/l	1,0
Hypernatraemia (↑ Sodium)	mEq/l	1,0	mmol/l	1,0
Hypokalaemia (↓ Potassium)	mEq/l	1,0	mmol/l	1,0
Hyperkalaemia (↑ Potassium)	mEq/l	1,0	mmol/l	1,0
Hypoglycaemia (↓ Glucose)	mg/dl	0,0555	mmol/l	18,0
Hyperglycaemia (↑ Glucose)	mg/dl	0,0555	mmol/l	18,0
Hypocalcaemia (↓ Calcium)	mg/dl	0,2495	mmol/l	4,0
Hypercalcaemia (↑ Calcium)	mg/dl	0,2495	mmol/l	4,0

[a] No SI unit

For example: Haemoglobin 9,5 g/dl - multiply by factor 0,6206 → 5,9 mmol/l

Appendix 4: Table with the used antibacterial and antifungal drugs used in the 6 participating Intensive Care Units.

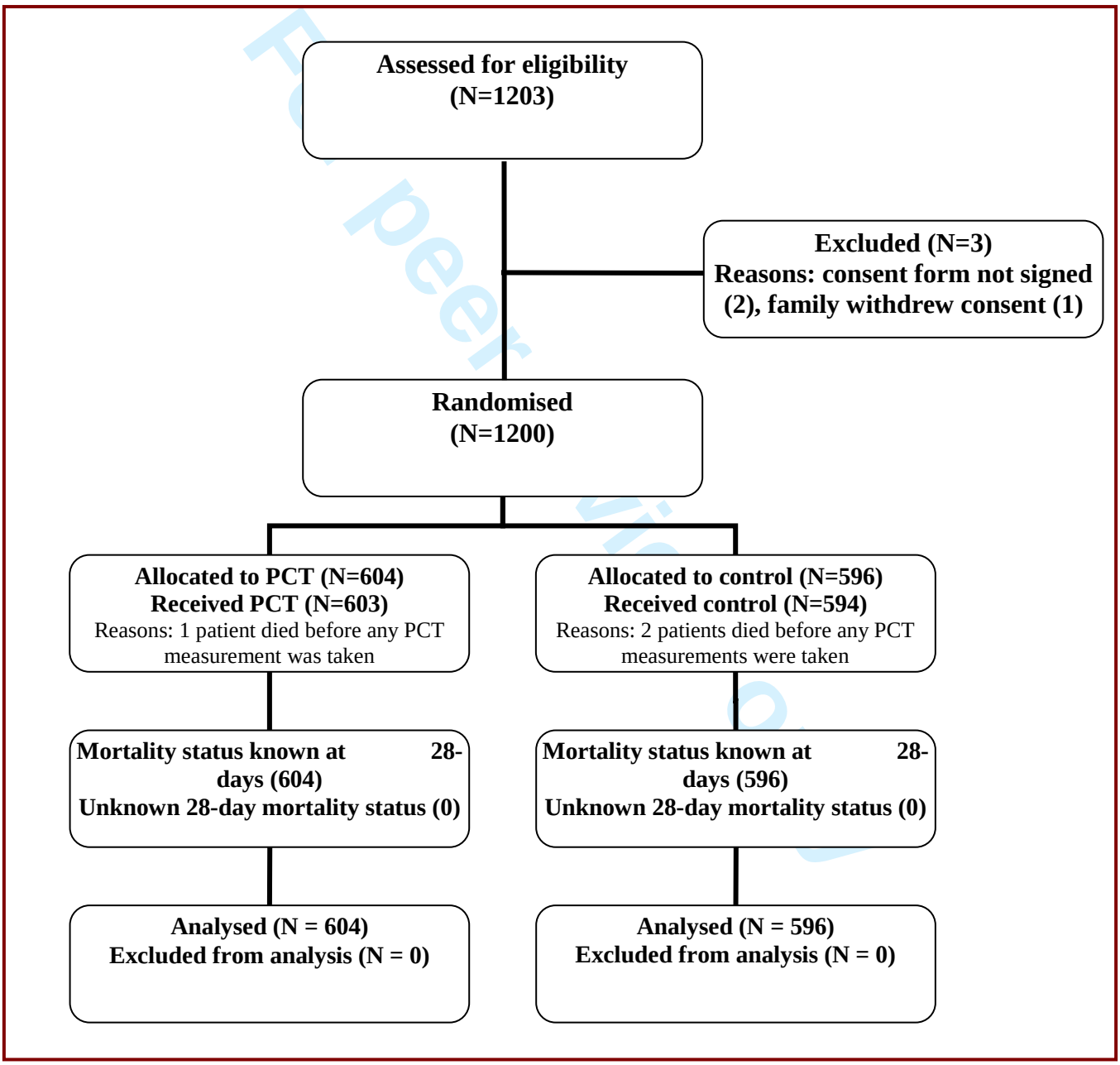
Generic name	Comercial name (s)
Benzyl-Penicillin	Penicillin"Leo", Penicillin"Rosco" Benzyl-Penicillin"Panpharma"
Phenoxymethyl-Penicillin	Calcipen ®, Pancillin ®, Primcillin ®, Rocilin ®, Vepicombin ®"DAK"
Dicloxacillin	Dicillin ®, Diclocil ®
Flucloxacillin	Heracillin
Amoxicillin	Amoxicillin"NM", Flemoxin Solutab ®, Imacillin ®, Imadrax ®,
Amoxicillin+Clavulanic Acid	Bioclavid, Bioclavid Forte, Spektramox ®
Ampicillin	Ampicillin"Vepidan", Doktacillin, Pentrexyl ®
Piperacillin	Ivacin ®, Pipril
Piperacillin+Tazobactam	Tazocin ®
Pivampicillin	Pondocillin ®
Pivmecillinam/ Mecillinam	Selexid ®
Cefalexin	Keflex ®
Cefalotin	Keflin ®
Cefepim	Maxipime ®
Cefotaxim	Claforan ®
Ceftazidim	Fortum ®
Ceftriaxon	Rocephalin ®
Cefuroxim	Zinacef, Cefuroxim Stragen, Zinnat ®
Aztreonam	Azactam ®
Meropenem	Meronem ®
Imipenem+cilastatin	Tienam ®
Azithromycin	Zitromax ®
Clarithromycin	Klacid ®, Klacid ® Uno, Klaricid, Zeclar
Erythromycin	Abboticin ®, Abboticin ® Novum, Erycin ®, Escumycin, Hexabotin ®
Roxithromycin	Surlid ®, Forimycin ®, Roximstad, Roxithromycin"Copyfarm", Roxithromycin"UNP"
Doxycyclin	Vibradox ®
Lymecyclin	Tetralysal ®
Oxytetracyclin	Oxytetral ®
Tetracyclin	Tetracyclin"AL", Tetracyclin"DAK", Tetracyclin"SAD"

Gentamicin	**Garamycin ®, Gentacoll ®, Hexamycin, Septopal, Septopal Mini**
Netilmicin	**Netilyn**
Tobramycin	**Nebcina ®, Tobi ®**
Moxifloxacin	**Avelox**
Ciprofloxacin	**Ciproxin ®, Cifin, Ciprofloxacin"1A Farma", Ciprofloxacin"2K Pharma", Ciprofloxacin"Alpharma", Ciprofloxacin"Biochemie", Ciprofloxacin"Gea", Ciprofloxacin"Ratiopharm", Sancipro, Sibunar ®**
Ofloxacin	**Tarivid ®**
Norfloxacin	**Zoroxin ®**
Methenamin	**Haiprex**
Nitrofurantoin	**Nitrofurantoin"DAK", Nitrofurantoin"SAD"**
Sulfamethizol	**Lucosil ®, Sulfametizol"SAD", Sulfametizol"Ophtha"**
Trimethoprim	**Monotrim ®, Trimethoprim"1A Farma", Trimopan**
Sulfamethoxazol+Trimethoprim	**Sulfamethoxazol+Trimethoprim"SAD", Sulfotrim ®**
Clindamycin	**Dalacin ®**
Colistin	**Colimycin**
Teicoplanin	**Targocid ®**
Vancomycin	**Vancocin, Vancomycin"Abbott", Vancomycin"Alpharma"**
Fusidinsyre	**Fucidin ®**
Linezolid	**Zyvoxid ®**
Metronidazol	**Flagyl ®, Metronidazol"Alpharma", Metronidazol"DAK", Metronidazol"SAD"**
Amphotericin B	**Abelcet, AmBisome, Fungizone**
Caspofungin	**Cancidas ®**
Fluconazol	**Conasol, Diflucan ®, Fluconazol"Alpharma", Fluconazol"Copyfarm", Fluconazol"Nycomed", Fluconazol"Ratiopharm", Fluconazol"Stada", Fungal ®, Fungustatin**
Flucytosin	**Ancotil**
Ketoconazol	**Nizoral ®**
Voriconazol	**Vfend**
Ethambutol	**Myambutol ®**
Isoniacid	**Isoniacid"OBA"**
Pyrazinamid	**Pyrazinamid"Medic", Pyrazinamid"SAD"**
Rifabutin	**Rifabutin"Pharmacia"**
Rifampicin	**Rimactan ®**

PASS-II 25th Aug 2010

Antibiotics and Renal Organ Failure – secondary endpoints from the Procalcitonin And Survival Study - analysis plan

1. Consort Flow Diagram (done in PASS-1)



Trial profile.

2. Baseline characteristics

Table 1: Baseline characteristics

	Standard-of-care-only n=596)	Procalcitonin-guided n=604)	Overall n=1200)
Age (Yr.) Median (IQR)	67 (58–75)	67 (58–76)	67 (58–76)
Male sex – no. (%)	333 (55·9%)	330 (54·6%)	663 (55·3%)
Body Mass Index – Median kg/m2 (IQR)	24·7 (22·0–27·8)	25·0 (22·5–28·7)	24·8 (22·2–27·9)
APACHE II Score - Median (IQR)	18 (13–24)	18 (13–25)	18 (13–24)
Surgical patient – no. (%)	260 (43·6)	227 (37·6)	487 (40·6)
Chronic co-morbidity* - no. (%)			
No chronic co-morbidities	102 (17·1)	123 (20·4)	225 (18·8)
1 chronic co-morbidities	279 (46·8)	257 (42·6)	536 (44·7)
2 chronic co-morbidities	173 (29·0)	171 (28·3)	344 (28·7)
≥3 chronic co-morbidities	42 (7·1)	53 (8·8)	95 (7·9)
Acute illness/reason for admittance to ICU – no. (%)			
Central nervous system incl. Unconsciousness	78 (13·1)	101 (16·7)	179 (14·9)
Respiratory failure	422 (70·8)	410 (67·9)	832 (69·3)
Circulatory failure	263 (44·1)	257 (42·6)	520 (43·3)
Gastro-intestinal disease	128 (21·5)	96 (15·9)	224 (18·7)
Renal disease	81 (13·6)	103 (17·1)	184 (15·3)
Post-operative complications	123 (20·6)	106 (17·6)	229 (19·1)
Trauma	113 (19·0)	106 (17·6)	219 (18·3)
Other	68 (11·4)	57 (9·4)	125 (10·4)
Indicators of severity			
Temperature, ^{0}C (median (IQR), n=1136)	37·3 (36·3–38·1)	37·4 (36·4–38·3)	37·3 (36·3–38·2)
Mean arterial pressure, mmHg (median (IQR) n=1195)	71 (60–84)	72 (63–85)	71 (62–84)
Heart frequency (median (IQR) n=1197)	100 (82–116)	100 (84–117)	100 (83–117)
Need for vasopressor/inotropic drug† (%, n=1200)	315 (52·9)	326 (53·4)	641 (53·4)
PaO2 /PaCO2 ratio (median (IQR), n=1178)	1·85 (1·27–2·62)	1·82 (1·29–2·53)	1·83 (1·28–2·59)
pH (median (IQR) n=1185)	7·29 (7·21–7·39)	7·29 (7·20–7·38)	7·29 (7·20–7·38)
Mechanical ventilation used (%, n=1200)	401 (67·3%)	401 (66·4%)	802 (66·8%)
Creatinine µmol/lL (median (IQR) n=1167)	119 (78–197)	119 (75–208)	119 (76–202)
Dialysis required (%, n=1200)	88 (14·8%)	86 (14·2%)	174 (14·5)
Bilirubin, µmol/L (median (IQR) n=1109)	10 (6–17)	10 (5–18)	10 (5–17)
Infection, clinical assessment ‡ – no. (%)			
No infection	118 (19·8)	86 (14·2)	204 (17·0)
Localized infection or Sepsis	266 (44·6)	271 (44·9)	537 (44·8)
Severe sepsis/ septic Shock	212 (35·6)	247 (40·9)	459 (38·3)
Site of infection § – no. (%)			
CNS	12 (2·0)	35 (5·8)	47 (3·9)
Respiratory	292 (50·0)	324 (53·6)	616 (51·3)
Gastrointestinal	149 (25·0)	145 (24·0)	294 (24·5)
Urinary	28 (4·7)	42 (7·0)	70 (5·8)
Other	52 (8·7)	41 (6·8)	93 (7·8)
Biomarkers			
Alert-PCT || – no. (%)	279 (47·0)	312 (51·7)	591 (49·4)
Leukocytes, x10^{9} – median (IQR)	13·0 (8·8–18·1)	12·4 (8·0–18·1)	12·8 (8·4–18·1)
C-reactive protein, mg/L – median (IQR)	152 (54–266)	161 (56–271)	157 (56–271)

Interquartile range (IQR). Acute Physiology and Chronic Health Evaluation II score (APACHE II) ranges from 0 to 71. *Chronic co-morbidity: Earlier diagnosed via hospital admission: heart failure, lung disease, cancer, diabetes, alcohol abuse, chronic infection, neurological disease, renal diseases, liver disease, gastro-intestinal disease, autoimmune disease, cancer and psychiatric disorders. Acute illness: persons can have several. 'Other' includes liver disease, haemorrhage, haematological disease and poisoning. †Vasopressors/inotropic drugs are considered to be epinephrine, nor-epinephrine, dopamine and dobutamine. ‡ Infections were rated according to the ACCP/SCCM definitions; investigators were trained in using them. § Site of infection: patients can have more than one. ||Alert-PCT: Procalcitonin-level not decreasing by at least 10% from the previous day and above 1·0 ng/ml. If only one measurement is available: Absolute procalcitonin-level above 1·0 ng/ml.

Table 1. Baseline characteristics of the study participants.

Table 2: Follow up characteristics

Follow up measurement	Control group (N=596)	PCT-guided group (N=604)	Overall (n=1200)
Patients followed and alive for 28 days (N., %)			
Patients followed for 28 days (incl. those who died in the first 28 days) (N., %)			
Status at 28 days (n =): Alive Dead			
Days spent in ICU Median (IQR) (as in PASS-I)			
Days spent in Danish hospital within 28 days Median (IQR)			
Patients with a complete 28 day follow up for respiratory failure (mech. Vent., PaO2 and FiO2)			
Days followed within 28 days for respiratory failure (mech. Vent, PaO2 and FiO2) of total days in trial ((denom. = 604 x 28) this can be drawn from the admission list in combination w. database)			
Patients with 28 day follow up for renal failure (dialysis – same as prev.)			
Days followed within 28 days for renal failure (dialysis) of total days in trial (denominator = 604 x 28 and 596 x 28 days) (same as prev.)			
Patients with 28 day follow up for renal failure (eGFR)			
Days followed within 28 days for renal failure (eGFR) of total days in trial (denominator = 604 x 28 and 596 x 28 days)			
Patients with 28 day follow up for Platelets			
Patients with 28 day follow up for Bilirubin			
Patients with 28 day follow up for antibiotic consumption			

n*s refers to the total number of patients who had follow up for 28 days.
28-day follow up is: Follow up until death within 28 days OR until day 28. For respiratory failure follow up is done for all ICU admissions. For renal failure, follow up is done for all dialysis treatment (ICU+other dialysis competent hospital units) and for all creatinine and carbamide measurements performed within 28 days (ICU + non-ICU admissions). For platelets and bilirubin, follow up is done for all measurements performed within 28 days (ICU + non-ICU admissions)

STRATIFICATION (*S) / test for interaction: (regarding the below analyses in Section 2 + 3)

1. **Age (limit initially 65 y, if significant interaction, more age groups**
2. **APACHE II score (limit initially 20, if significant interaction, more APACHE II groups,**
3. **Site 1-9.**
4. **Severe Sepsis/septic Shock vs. Milder or No infection at Baseline**
5. **Calendar date of inclusion into PASS. Recruited: 9th Jan 2006 – 31st December 2007 (~430 patients) vs. 1st of Jan 2008 – 2nd of June 2009 (~770 patients).**
6. **Surgical patient / medical patient [Surgical = All patients with mark in Baseline "B6", or "B12" or marked "Yes" in "L"]**
7. **Gender**

SECTION 2. Exposure – Antibiotic usage

Follow up: All patients were followed up regarding antibiotic consumption: 1) In the ICU in the primary PASS-CRF, 2) All ICU-surviving patients, not staying in the ICU for 28 days, were followed up for antibiotic consumption in the non-ICU, they were discharged to after ICU.

General: The aims of these analyses are to investigate the impact of performing PCT-guided empiric antibiotic interventions according to a progressive algorithm on the consumption of antibiotics. This is to be illustrated by analyses exploring 1) spectrum, 2) quantity and 3) duration of therapy in the two arms.

The aim is:

a) To investigate the difference in exposure in general to antibiotics in the two arms of the PASS trial and more specifically to broad-spectrum antibiotics.

This is done in the following analyses (PCT vs. Control):

1) The total number of days within the 28 day follow-up period with any antibiotic treatment (or proportion of follow-up time): [Not done Yet]
2) The total consumption of any antibiotic in weight (grams within 28 days) [Not done Yet]
3) The total consumption per ICU day of any antimicrobial [DONE]
4) The total consumption of betalactam drugs active against most Extended Spectrum Beta-lactamases and wild-type Pseudomonas aeruginosa (a. Meropenem and other pseudomonas active carbapenems, OR b. Piperacillin/tazobactam OR c. 4.generation Cephalosporins). [or proptortion of days in these treatments] [Not done Yet]
5) The total no. of days within the 28-day follow up period with treatment with any flour-quinolone (ciprofloxacin, moxifloxacin and others) [or proptortion of days in these treatments] [Not done Yet]
6) The total no. of days within the 28-day follow up period with treatment with any glycopeptide (Vancomycin, Teicoplanin) [or proptortion of days in these treatments] [Not done Yet]
7) The total no. of days within the 28-day follow up period with treatment with fluconazole [or proportion of days in these treatments] [Not done Yet]

Consumption of antimicrobials in the intensive care unit				
Length of antimicrobial treatment in ICU, days (median, IQR)	4 (3– 10)	6 (3– 11)	-	0·001
Quantity of antimicrobials administered per ICU day (g) (median, IQR)	6·7g (4·5g– 12·5g)	8·6g (5·3g– 13·7g)	-	<0·001
Number (%) ICU days spent with at least three antimicrobials	2721 (57·7%)	3570 (65·5%)	-7·9% (-9·7%– -6·0%)	0·002

*Counted from the time of sampling. Only samples later to become positive. Cultures with coagulase negative staphylococci, corynebacteria and propionebacteria are not included. † Including localised infection, mild sepsis, severe sepsis and septic shock. p-values for the number of days spent with each factor were generated by testing the proportion of intensive care days spent with each factor using non-parametric tests. ICU: Intensive care unit

Table 3. Antibiotic consumption

Admission time within 28 days

1. Number of days admitted to hospital within 28 days after recruitment. Median + IQR. (PCT vs. Control)

Subgroup Analysis: Total use of Antimicrobial chemotherapy

1. Total antibiotic prescription days (all AMCs received, where all AMCs are weighted equally and summed per day, e.g.:→ possible to have e.g. 30 prescription days in 10 days ICU)

Table 3: Number of AMCs received per day (over all days)

	PCT-arm	Control-arm	P-value
AMC total (N,. %)			
Recruited 09/01/06 – 31/12/07 Recruited 01/01/08 – 02/06/09			
Age <65 years Age ≥65 years			
APACHE II <20 APACHE II ≥20			
Bispebjerg Gentofte Glostrup Herlev Hillerød Hvidovre Roskilde Skejby Århus			
Severe Sepsis or septic shock at BL Milder or no infection at BL			
Surgical patient Non-surgical patient			
Gender			

MICROBIOLOGY

Follow up: All patients were followed up via the electronic registers at the microbiologic depts., who service the PASS-ICU´s regarding all microbiologic samples performed from baseline and until 28 days after. Data have been merged in the PASS-database.

Table 4: Number of culture samples performed within 28-days from randomisation [Not done Yet – JU handles this]

Intervention		PCT arm N =	Control Arm N =	P-value
Microbiology:	**N., (%)**			
Blood Cultures	N. Yes, (%)			
Urine Cultures	N. Yes, (%)			
Airway Cultures	N. Yes, (%)			
Samples from other foci	N. Yes, (%)			

SECTION 3a: Estimating the degree of Organ Failure (OF)

Follow up: All patients were followed up regarding respiratory failure (mech. Vent + physiologic parameters) and renal failure at 1) the PASS-ICU where the patient was recruited in the primary PASS-crf, 2) regarding mech. Ventilation and physiologic parameters and renal failure at any other PASS-ICU within the 28 day period (when patients were discharged to such an ICU, 3) in the case that a patient was discharged within the 28 day period to a non-PASS ICU (seldom), follow up was made for mech. Vent. and physiologic parameters and renal failure in hospitals "Rigshospitalet" and "Bispebjerg", since only very few ICU days were spent at any other ICU within the 28 day period (48 days of approx 9900 days = approx 0.5%).

The purpose of these analyses is to explore in detail, the quantity of the occurrence of secondary endpoints in the PASS-trial, especially respiratory organ failure and renal organ failure.

Genuine hypothesis: High usage of broad spectrum antibiotics as used in the PASS trial, results in substantially reduced organ function (respiratory, renal and liver) and compromised coagulation and a likewise substantially increased time with manifest organ failure as defined clinically (need for organ support) AND biochemically/fysiologically (measured objective parameters).

NB: Analyzes are summarized in the table 5 below

time)

A. Renal Failure:

a. Median/ Mean eGFR for day1 – day10
b. Median/ Mean eGFR for day11 – day28
c. Median/Mean eGFR for day1 – day28 (a+b) [eGFR on days in columns in a figure and AUC for the columns]
d. Median/Mean Carbamide for day1- day10
e. Median/ Mean Carbamide for day11 – day28
f. Median/Mean Carbamide for day1 – day28 (a+b) [Carbamide level on days in columns in a figure and AUC for the columns]
g. Median/Mean Platelet count for day 1-28 [[platelet on days in columns in a figure and AUC for the columns]
h. Median/Mean Bilirubin [Bilirubin on days in columns in a figure and AUC for the columns]
i. No. of days within 28 days with eGFR < 60 ml/min/1.73 m2
j. No. of days within day1 – day10 with eGFR < 60 ml/min/1.73 m2
k. No. of days within day1 – day10 with dialysis
l. No. of days within day11 – day28 with dialysis
m. No. of days within day1 – day28 with dialysis

C + F+ G + H are all part of one figure with 4 panels.

Explanations: A: Dialysis:

Patients are categorized on days with ND or NA as dialysis=0, since this means patient has been discharged to home. All admissions within 28 days have been drawn from the central hospital register (Green System) and all admissions at dialysis capable departments have been followed up with dialysis.

B: eGFR:

In the ICU, patients are categorized with a new eGFR every day (done in PASS).

Patients are categorized on the basis of their status of eGFR on the last day of ICU. This status is kept until a creatinine measurement is done (on which day the status is changed to a new eGFR). This status is then kept until the next time creatinine is measured – and so forth. In this way every day from 1 – 28 is given an eGFR status.

In summary, the same principle is used: From day 1, the first time a creatinine is measured, a eGFR is calculated. Next time the patient has a creatinine measurement, the patient is re-categorized with a new eGFR. That eGFR is kept until the next creatinine measurement etc.

Table 5. Prevalence and duration of organ failure and other severe disturbances (PCT vs. Control)

	PCT arm (n =)	Control Arm (n =)	P-value
Kidney Failure mL/min/1.73 m^2 (N. days, % of total days): Normal: GFR > 90 Mildly impaired: 60–89 Moderately/severely impaired: GFR <60			
Kidney Failure Median/ Mean eGFR for day1 – day10			
Kidney Failure Median/ Mean eGFR for day11 – day28			
Kidney Failure Median/Mean eGFR for day1 – day28 (a+b)			
Kidney Failure Median/Mean Carbamide for day1- day10			
Kidney Failure Median/ Mean Carbamide for day11 – day28			
Kidney Failure No. of days within 28 days with eGFR < 60 ml/min/1.73 m2			
Kidney Failure No. of days within day1 – day10 with eGFR < 60 ml/min/1.73 m2			
Kidney Failure No. of days within day1 – day10 with dialysis			
Kidney Failure No. of days within day11 – day28 with dialysis			
Kidney Failure No. of days within day1 – day28 with dialysis			

Table with summarized analyses.

SECTION 3b: Attempting to explain the reason for organ failure (if OF is confirmed in section 3a)

Antimicrobial toxic explanation

Genuine hypotheses:

1) High Exposure (at least 5 or at least 10 days) to a certain combination of antibiotics (Pip/Tazo+Cipro OR Meropenem + Cipro OR Pip/Tazo + Vanco OR Meropenem + Vanco) causes OF

For 2-6: Estimate accumulated risk for day 1, 2, 3 etc. separately in both PCT group and control group.

2) Treatment for more than 4 days with Pip/Tazo causes OF (also 10 days)
3) Treatment for more than 4 days with Ciprofloxacin causes OF (also 10 days)
4) Treatment for more than 4 days with Meropenem causes OF (also 10 days)
5) Treatment for more than 4 days with Vancomycin causes OF (also 10 days)
6) Treatment for more than 4 days with Cefuroxim causes OF (also 10 days)

For the below analyses two composite endpoints are used for the Pulmonary/renal OF:

1) **Organ failure endpoint A**: Clinical Organ Failure judgment: Endpoint=1 for any day with dialysis. If both are present, Endpoint=2. Results are presented as “Clinical Organ Failure Days”
2) **Organ failure endpoint B:** Objective Organ failure measures: Endpoint =1 for any day with eGFR <30, repeated with <60 ml/min/1,73 m2. “Objective Organ Failure Days”

Analyses:

A. Objective Organ failure endpoint:

As above, 1) – 6).

1) Analyze the median “Objective Organ Failure Days” to occur from “P-T treatment day 5” until 10 days later (counting endpoints for next 10 days). Censor at death.
2) Analyze the median “Objective Organ Failure Days” to occur from “Meropenem treatment day 5” until 10 days later (counting endpoints for next 10 days). Censor at death

B. Multiple Effects models:

Regarding renal dysfunction: Analyze renal recovery in eGFR progression per day on different drugs day 1-10 (Meropenem / Piperacillin-tazobactam / Ciprofloxacin / Cefuroxim), control for other known predictors of renal failure. Additionally after discontinuation of these drugs.

Sensitivity analyzes:

Cox or Logistic Regression ?

Endpoint: Binary endpoint. To be defined according to the median number of organ failure days within 10 days after exposure for 5 days.

Endpoint 1a: [>median number of “clinical organ failure days”]

Endpoint 1b: [>median number of “clinical organ failure days”+2 days]

Endpoint 2a: [>median number of “objective organ failure days”]

Endpoint 2b: [>median number of “objective organ failure days”+2 days]

Risk variables to be entered:

a. Treatment for >=4 days with Pip/tazo

b. Treatment for >=4 days with Meropenem

c. Treatment for >=4 days with Ciprofloxacin

d. Treatment for >=4 days with Vancomycin

e. Treatment for >=4 days with Pip/tazo + Ciprofloxacin (all 4 days)

f. Treatment for >=4 days with Meropenem + Ciprofloxacin (all 4 days)

g. Treatment for >=4 days with Pip/tazo + Vancomycin (all 4 days)

h. Treatment for >=4 days with Meropenem + Ciprofloxacin (all 4 days)

i. Treatment for >=4 days with Meropenem + Vancomycin (all 4 days)

j. APACHE II >=20

k. Age >=65

l. Surgical patient

m. Severe sepsis/septic shock

NB: Treatment count start days 1 – 13 (so 5 days complete on day 5 – 18).

Patients with pauses in the administration of >=1 day → exclude

Only count the first administration

Endpoints:

“Clinical Organ Failure Days” and “Objective Organ Failure Days” both as defined above

→Transformed to Binary endpoint:

Endpoint 1a: [>median number of “clinical organ failure days”]

Endpoint 2a: [>median number of “objective organ failure days”]

(as above in the sensitivity analysis)

PASS-II, organ failure – authors,

Forfattere

Chip: JU+JDL+LRN

KMA Hvh/Diacenter: BEL

Glostrup: Mulige: Asger, Anne, Ditte

Hvh: Mulige: Peder C, Jesper, Morten

Herlev: Mulige: Peter, Hamid, Tina

Gentofte: Mulige: Thomas, Katrin

Hillerød: Mulige: Morten, Lars, Kristian A?

Roskilde: Mulige : Niels-Erik

Århus: Mulige: Kim + Mads

Skejby: Mulige: Paul

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts[21 31])	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	4
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	1,5,15
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6 + fig. 2 + Diagram D1
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	7-8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5

Section/Topic	Item No	Checklist item	Reported on page No
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	6
	11b	If relevant, description of the similarity of interventions	6
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	6-7
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	6-7
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	Figure 1 (CONSORT diagram
	13b	For each group, losses and exclusions after randomisation, together with reasons	Figure 1 (CONSORT diagram
Recruitment	14a	Dates defining the periods of recruitment and follow-up	8
	14b	Why the trial ended or was stopped	8
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	8-9, table 3 +table 4
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect	

Section/Topic	Item No	Checklist item	Reported on page No
		size and its precision (such as 95% confidence interval)	9-10 + table 2, 3, 4 + fig. 3+4
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Abstract + p.
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	Table 3, fig. 3+4, p 10.
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms[28])	Table 3+4, p. 10-11, fig. 3+4
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	10-14
Other information			
Registration	23	Registration number and name of trial registry	4-5
Protocol	24	Where the full trial protocol can be accessed, if available	5
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration[13] for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials,[11] non-inferiority and equivalence trials,[12] non-pharmacological treatments,[32] herbal interventions,[33] and pragmatic trials.[34] Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

Kidney failure related to broad-spectrum antibiotics in critically ill patients: secondary end point results from a 1200 patient randomized trial

Corresponding author Jens-Ulrik Jensen, Copenhagen HIV Programme, The Panum Institute, Faculty of Health Sciences, University of Copenhagen, Blegdamsvej 3B, DK-2200 Copenhagen N, juj@cphiv.dk

Jens Ulrik Jensen *medical doctor*[1,2], Lars Hein *anaesthetist*[3,4], Bettina Lundgren *centre director, hospital diagnostic centre* [2,5], Morten Heiberg Bestle *anaesthetist* [4], Thomas Mohr *anaesthetist*[6], Mads Holmen Andersen *anaesthetist*[7], Klaus Julius Thornberg *anaesthetist*[6], Jesper Løken *anaesthetist*[8], Morten Steensen *anaesthetist*[8], Zoe Fox *biostatistician*[1,9], Hamid Tousi *anaesthetist*[10], Peter Søe-Jensen *anaesthetist*[10,] Anne Øberg Lauritsen *anaesthetist*[3], Ditte Gry Strange *anaesthetist*[3], Nanna Reiter *anaesthetist* [11], Katrin Thormar *anaesthetist*[6], Paul Christian Fjeldborg *anaesthetist* [7], Kim Michael Larsen *anaesthetist*[12], Niels-Erik Drenck *anaesthetist*[11] Maria Egede Johansen *junior research associate*[1], Lene Ryom *junior research executive*[1], Christian Østergaard *senior research executive*[2,13], Jesper Kjær *database manager*,[1] Jesper Grarup *administrative leader* [1], Jens D. Lundgren *professor of infectious diseases*,[1,14] of the The Procalcitonin And Survival Study (PASS) Group*.

[1]Copenhagen HIV Programme at the University of Copenhagen; [2]Department of Clinical Microbiology at Copenhagen University Hospital Hvidovre; [3]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Glostrup; [4]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Hillerød; [5]Diagnostic Centre at Copenhagen University Hospital Rigshospitalet; [6]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Gentofte; [7]Department of Anesthesia and Intensive Care at Aarhus University Hospital in Skejby; [8]Department of Anesthesia and Intensive Care at Copenhagen University Hospital Hvidovre; [9]Royal Free Hospital School of Medicine in London; [10]Department of

Anesthesia and Intensive Care at Copenhagen University Hospital Herlev; [11]Department of Anesthesia and Intensive Care at Copenhagen University Hospital in Roskilde; [12]Department of Anesthesia and Intensive Care at Aarhus University Hospital in Aarhus; [13]Department of Clinical Microbiology at Copenhagen University Hospital Herlev; [14]Department of Infectious Diseases at Copenhagen University Hospital Rigshospitalet. All except[9] are from Denmark. [9] is from England.

*Participating investigators are listed in the appendix.

Running Title: Broad-Spectrum Antibiotics and Renal Failure in Critically Ill Patients

Keywords: Antibiotics – Renal Failure – Sepsis – Intensive Care

Abstract

Objectives: To explore whether a strategy of more intensive antibiotic therapy leads to emergence or prolongation of renal failure in intensive care patients.

Design: Secondary analysis from a randomized antibiotic strategy trial (the PASS study). The randomized arms were conserved from the primary trial for the main analysis.

Setting: Nine mixed surgical/medical intensive care units across Denmark.

Participants: 1200 adult intensive care patients, 18+ years, expected to stay +24 hours. Exclusion criteria: Bilirubin >40 mg/dL. Triglycerides >1000 mg/dL, Increased risk from blood sampling, pregnant/breast feeding and psychiatric patients.

Interventions: Patients were randomized to: guideline-based therapy ('standard-exposure'-arm), or to guideline-based therapy supplemented with antibiotic escalation whenever procalcitonin increased on daily measurements ('high-exposure'-arm).

Main outcome measures: Primary endpoint: estimated GFR<60 ml/min/1.73 m2. Secondary endpoints: a) delta eGFR after starting/stopping a drug, b) RIFLE criterion *Risk* "R", *Injury* 'I' and *Failure* 'F'. Analysis was by intention to treat.

Results: 28-day mortality was 31.8% and comparable (Jensen et al, CCM 2011). A total of 3672/7634 (48.1%) study days during follow-up in the 'high-exposure' vs. 3016/6949 (43.4%) in the 'standard-exposure'-arm were spent with eGFR <60 ml/min/1.73m2, p<0.001. In a multiple effects model, piperacillin/tazobactam was identified as causing the lowest rate of renal recovery of all antibiotics: 1.0 ml/min/1.73 m2 per 24h while exposed to this drug [95% CI: 0.7 – 1.3 ml/min/1.73 m2/24h] vs. meropenem: 2.9 ml/min/1.73 m2/24h [2.5 – 3.3 ml/min/1.73 m2/24h]); after discontinuing piperacillin/tazobactam, the renal recovery rate increased: 2.7 ml/min/1.73 m2 /24h [2.3 – 3.1 ml/min/1.73 m2 /24h]). eGFR<60 ml/min/1.73m2 in the two groups at entry and at last day of follow-up was 57% vs. 55% and 41% vs. 39%, resp.

Conclusions: Piperacillin/tazobactam was identified as a cause of delayed renal recovery in critically ill patients. This nephrotoxicity was not observed when using other beta-lactam antibiotics.

Trial registration ClinicalTrials.gov identifier: NCT00271752.

Introduction

Frequent complications to sepsis are organ failure, especially respiratory failure and renal failure [1-3]. Critically ill patients are more vulnerable to organ-related drug toxicities than less severely ill patients[4]. Randomized trials assessing safety of broad-spectrum antibiotics in intensive care settings are generally scarce, do not have sufficient statistical power for assessing organ failure endpoints, and do often not include defined kidney organ failure endpoints[5-7]. Data on renal failure endpoints are also sparse in the published trials from other patient populations, and since the absolute risk of renal failure is low for these patients, analyses may likely have been underpowered[8-12].

To our knowledge, randomized trials comparing 'high exposure' vs. 'standard exposure to antibiotics' and specifically addressing whether these interventions affect the occurrence and duration of kidney failure have not been done before in intensive care settings.

In this secondary analysis from a randomized trial, the PASS study[13], we aimed to explore whether a strategy of more intensive antibiotic therapy leads to adverse renal outcomes within 28 days after recruitment.

In our study population (and often in severely infected ICU patients), a bacterial hit has resulted in acute onset renal failure, and this bacterial hit (and related organ failure) is often the reason for ICU admittance. In such situations, with the correct treatment of the underlying infection, we expect renal function to recover. "Lack of recovery" is a non-desirable situation, which may be very serious for the patient. We wanted to explore this, and realizing, RIFLE/AKIN could not capture

this, we have used eGFR<60 ml/min/1.73 m^2 as the primary endpoint and examined this from different angles (eGFR<60 ml/min/1.73 m^2 at day 7, days with ml/min/1.73 m^2 . The multiple effects model was built to capture actual estimates of renal function improvement using different antibiotics and adjusting for other known or suspected causes of renal dysfunction.

Secondly, if renal failure was observed from the 'high exposure' approach, to identify one or several of the antibiotics used in this trial as the cause of such a renal failure.

Methods

Trial design and participants

PASS is a multicentre randomized controlled trial in Denmark 2006-9 in 1200 adult critically ill patients, expected to stay in one of the nine participating mixed medical/surgical intensive care units ≥24 hours; the CONSORT trial diagram is displayed in supplementary figure 1. Patients were randomized 1:1 either to treatment according to international guidelines: 'standard exposure arm', or to same guidelines but supplemented with daily drug-escalation initiated upon procalcitonin increases ('high exposure'-arm); 28-day mortality was 31.8% and comparable between the two groups, as reported[13].

To be eligible, patients had to be ≥18 years, enrolled within 24 hours of admission to the intensive care unit and have an expected intensive care-admission length of ≥ 24 hours. Patients with known bilirubin >40 mg/dL and triglycerides >1000 mg/dL (not suspensive) were not eligible (interference with procalcitonin measurements), as were patients who were judged to be at an increased risk from blood sampling. The inclusion criteria were broad since infection is frequent and often causes complications in the patient group and to increase the external validity of the results. The person or next of kin gave informed consent. The study protocol was approved by the regional ethics committees in Denmark (H-KF-272-753) and adheres to the Helsinki declaration, revised in Seoul 2008.

In the present analyses we explored presence and duration of renal failure as well as change in renal function during the observed time. Endpoints are defined in *statistical analysis* below. Patients were followed until day 28. The primary trial protocol and the analysis plan is available in the online supplement. Analysis was by intention to treat: NCT00271752.

Randomization and masking

Randomization was performed 1:1 using a computerized algorithm created by the database manager (JK) with concealed block-size, pre-stratified for site of recruitment, initial APACHE-II and age (entered in an encrypted screening form in a password protected website); investigators were masked to assignment before, but not after, randomization. All investigators were trained by the coordinating centre and had to register in an investigator-database. Investigators, treating physicians and the coordinator were unaware of outcomes during the study, as were they of all procalcitonin measurements in the 'standard exposure' (control)-group.

Antibiotic therapy in the two arms

The investigators enrolled participants and assigned the 'high exposure group' participants to the intervention. In the 'standard exposure' group, the antimicrobial treatment was guided according to current clinical guidelines[14], based on clinical assessment, microbiology and radiology among other parameters, as described elsewhere[13]

In the 'high exposure' group, the use of antimicrobial interventions was guided by the same clinical guidelines as in the 'standard exposure' group to ascertain the best standard of care therapy for all patients, and additionally antimicrobial interventions were initiated whenever procalcitonin levels were not decreasing at a pre-defined pace (supplementary figure 2) and diagram D1 in the online supplement where a site-adjusted local guideline is displayed.

Measurements, data collection and follow-up

Blood samples for biomarker measurement were made daily in the intensive care unit, beginning immediately after randomization. The assay used was the Kryptor®-PCT. Organ failure and antibiotic exposure was followed up for until 28 days or death, as described[13]. Mortality was followed via the National Patient Register in which all deaths in Denmark are registered within 14 days. Good Clinical Practice guidelines were applied. The regional ethics board approved the protocol (H-KF-01-272-753).

Statistical analysis

The primary endpoint was 'estimated GFR<60 ml/min/1.73 m^2' and several analyses were made to explore this: 'days with estimated GFR<60 ml/min/1.73 m^2', 'risk of estimated GFR<60 ml/min/1.73 m^2 on day 1-7'. Secondary endpoints were a) delta eGFR after starting/stopping a drug, b) RIFLE-criteria *Risk* 'R'. , *Injury* 'I' and *Failure* 'F' www.adqi.net. Since we explored exposure of antibiotics from baseline and forth (and not pre-ICU), in the RIFLE definition, the baseline creatinine was used (instead of an ideal eGFR). eGFR was calculated for every day. To not let this be influenced by hydration status, the baseline weight was used, and thus the relation between se-creatinine and eGFR was a first degree function for every patient. Other endpoints explored were 'ever' blood-urea level ≥20 mmol/L and eGFR<30.

The multiple effects eGFR 'slope' analyses, were adjusted for the following variables: treatment arm ('high exposure' vs. 'standard exposure'), age (≥65 vs. <65 years), gender, baseline APACHE II score (≥20 vs. <20), degree of host response/infection at baseline (severe sepsis/septic shock vs. milder or no infection as defined[15]), the eGFR at initiation of the investigated antibiotic, and finally, whether the patient at baseline was considered to be 'surgical' or 'medical'.

Comparisons were made between treatment arms using Students t-tests (for normal distributed continuous data) and Mann-Whitney U-tests (for non-normally distributed continuous data). Chi-squared tests and logistic regression models were used to test categorical variables. Time-to-event

analyses comparing the 'high exposure' group with the 'standard exposure' group were performed using Kaplan-Meier plots and Cox proportional hazards models. Interactions were explored whenever an interaction could be rationally expected according to background literature, for the multivariate models performed. Statistical analyses were performed using STATA Version 10.2, and SAS version 9.1. All reported p-values are 2-sided using a level of significance of 0.05.

Sample size

A multivariate approach power calculation was made: The summed squared correlations (Σrho^2) to the risk of the antibiotic drug investigated, was set to 0.3. The frequency of the endpoint in the 'standard exposure' group was set to 20%, the sample size was set to 1200, and the frequency of the exposure was set at 30%, which resulted in a detection limit for odds ratio of ≥ 1.5 (or ≤ 0.67).

Results

Baseline characteristics

Nine sites included 1200 persons between 09/01/06 and 02/06/09. Eighty-three percent of the patients were assessed by the investigator to have an infection at baseline and 81% of the patients suffered from chronic co-morbidity. Supplementary table 1 briefly summarizes baseline characteristics. Mortality was comparable between the two groups, as reported[13].

Follow-up

Follow-up for renal measures during the 28-day study period was made on 9,348 days in the 'standard-exposure' group of 10,755 days alive and admitted to hospital (86.9%) vs. 9,866 of 11,380 days in the 'high exposure group' (86.7%). If time after discharge from hospital (where no S-creatinine values were determined) until day 28 was included, the percentage of days with assessment of renal failure was 71.2% (9,348/13,130 days) vs. 73.8% (9,866/13,377 days)."

Use of Antibiotics

The antibiotics used most while admitted to the ICU were piperacillin/tazobactam, cefuroxim, meropenem and ciprofloxacin, and there was a substantial higher use of piperacillin/tazobactam and ciprofloxacin in the 'high exposure' arm (supplementary table 2). Vancomycin was used to a lesser extent in both groups and aminoglycosides and colistin were used rarely in both groups.

The median length of an antibiotic course was prolonged using the 'high exposure'-algorithm (6 days (IQR 3, 11) vs. 4 days (IQR 3, 10), p=0.004.

Renal failure in the originally randomized study arms

The % of days within day 1-28 with eGFR≤ 60 ml/min/m^2 was 48% in the 'high exposure' arm vs. 43% in the 'standard exposure' arm, p<0.0001. Results in table 1 are estimated eGFR values, based on actual measured S-creatinine values; results regarding days with eGFR were comparable if using the 'last observation carried forward' approach (not shown). RIFLE-criterion 'R' occurred more often within day 1-28 in the 'high exposure' arm than the 'standard exposure' arm: 209 patients vs. 170 patients, p=0.02, as did blood urea levels exceeding 20 mmol/L: 253 (43.4%) vs. 217 (37.4%), p=0.04.

The frequency of renal failure on the last day of follow-up was comparable between the arms (table 2), underlining that the results depicted in table 1 reflect a temporary extension of duration of renal failure in the "high exposure group" and furthermore that this observation is not explained by premature discharge of renally incompetent patients in the 'standard exposure' arm.

Glomerular Filtration Rate changes and exposure to certain antibiotics

Comparison of the eGFR of all patients (both study arms) for the first ten days after starting on the most frequently used betalactam antibiotics showed that the slowest recovery of renal function was

observed in patients on piperacillin/tazobactam as compared to patients on meropenem or cefuroxim (figure 1). A multiple effects model investigating the eGFR regression coefficient ('increase in eGFR') per day on these drugs confirmed that renal recovery was lowest in patients on piperacillin/tazobactam (table 3). Of note, renal recovery seems to be low in patients exposed to cefuroxim, but as displayed in fig. 1, this drug is given to patients with a relatively normal renal function (leaving few possibilities for 'recovery').

For the first five days following discontinuation of these drugs, adjusting for the same variables, eGFR increased at the highest rate in patients receiving piperacillin/tazobactam (table 3).

The frequency of eGFR<60 ml/min/1.73 m^2 on day 7 (or at death or last follow-up day) in the trial was 523/1200 = 43.6%. This endpoint was investigated in a forward censored ($p<0.1$) logistic regression. Use of piperacillin/tazobactam and other frequently used beta-lactam drugs for at least three days within these first seven days, as well as known and suspected predictors of renal failure were explored in a multivariable logistic regression analysis. Five independent predictors of renal failure on day 7 were identified: Age above 65 years, APACHE II score >20, Charlson´s co-morbidity score ≥2, estimated GFR at baseline and use of piperacillin/tazobactam for at least 3 days within the first 7 days (table 4) Excluding all patients who died within the first seven days, excluding all patients with invasive fungal infection on day 1-28, combining the betalactam exposure with exposure to flour-quinolone exposure (data not shown) or 4) adding 'Alert-procalcitonin' at baseline as a variable, did not alter the signal (data not shown). To validate the endpoint as a predictor of mortality, a Cox regression was done; eGFR <60 mL/min/1.73 m^2 on day 7 was found to be the strongest predictor of 'all cause mortality day 7-28' of all tested variables (Table T1, supplementary material).

Discussion

Principal findings

We observed that the duration of renal failure is prolonged in critically ill patients randomized to receive high exposure to broad-spectrum antibiotics and escalated diagnostic work-up according to a biomarker-strategy, compared to patients randomized to receive standard care according to guidelines regarding use of antibiotics and diagnostics. This difference in renal function was mainly confined to a prolongation of existing renal dysfunction, since there was only a moderate, although significant, difference in de novo acute renal failure.

To our knowledge, this study provides the first clinical report to inform this critical issue within ICU medicine. Firstly, the study was a randomized, good clinical practice controlled trial with a high sample size for comparison of organ failure, and the patients' baseline characteristics in general and specifically regarding renal parameters, were comparable. Secondly, the rate of follow-up, although not complete for the entire period, was high and equal among the groups and the rate of renal failure on the last day of follow-up in the two groups was comparable. Thus, the observed increased risk of persistent renal failure in the "high-exposure group" is attributable to this intervention in some way.

The intervention consisted of an increased number of culture samples, a proposed initiative to do further diagnostic imaging (no observed difference) and a rapid and aggressive antibiotic escalation with certain drugs, which was documented to be of substantial extent (supplementary table 2). As a moderate increase in microbiologic sampling would not cause renal failure, and since there was no observed increase in diagnostic imaging, these interventions seems implausible reasons to explain the observations depicted in table 1.

This leaves us with the documented escalation in use of piperacillin/tazobactam and ciprofloxacin as possible explanations. Before concluding, that the observed renal dysfunction was caused directly by one (or both) of these drugs, we wanted to exclude the possibility that the results had appeared because of a derived effect of an increase in fungal infections. Fungal infections have been linked to broad-spectrum antibiotics[16], and renal failure is a well-known complication to some

antifungals[17]. However, excluding all patients with invasive fungal infections did not alter the results.

Based on these results, and after having excluded other potential explanations, we realized that nephrotoxicity from piperacillin/tazobactam and/or ciprofloxacin was the most plausible explanation of the observed renal dysfunction. To further substantiate this, several analyses were conducted. A multiple effects model was built to examine the GFR in the days after administration of different frequently used drugs. This model included the five most often administered antibiotics, including piperacillin/tazobactam, meropenem, cefuroxim, ciprofloxacin and vancomycin along with other known and suspected causes of renal failure. In this model, the use of piperacillin/tazobactam was associated with a striking low rate of GFR-improvement, compared to the other drugs investigated. Intriguingly, this adverse effect appears to be reversible, since patients in whom, piperacillin/tazobactam was discontinued, had the fastest improvement in renal function as compared with patients on other antibiotic courses. Several sensitivity analyses were performed with findings consistent with this observation.

Comparison with other studies

Although clinical evidence regarding renal failure according to use of piperacillin/tazobactam in ICU patients has been limited, the influence of piperacillin on renal function has been investigated in healthy volunteers in laboratory experiments. In a cross-over experiment, the influence on drug clearance from concurrent administration of piperacillin and flucloxacillin was estimated[18]. The authors observed that flucloxacillin clearance was reduced to 45% [90% CI: 40 – 50%] when piperacillin was administered simultaneously, whereas piperacillin clearance was unaffected by concurrent flucloxacillin administration. Time-clearance slope modeling identified competitive inhibition of renal tubular secretion as the most likely explanation. Piperacillin-induced reduction of imipenem clearance[19] and of tazobactam clearance has also been found[20], and a high correlation

between creatinin clearance and piperacillin clearance has been documented[21], and thus, it is plausible that piperacillin specifically causes nephrotoxicity.

Additionally, the published randomized trials comparing piperacillin/tazobactam with other beta-lactam drugs in intensive care unit settings are scarce, underpowered for assessment of renal failure endpoints and do generally not address renal endpoints[5-7]. Trials from other settings: haematological patients, diabetes patients, and surgical settings do generally not investigate renal failure endpoints, and in the few (non-ICU) trials that do report kidney endpoints, the total frequency of these makes the power to avoid type II error very low (diagram D2, online supplement).

Strengths and weaknesses of the study

Although our study is performed on analyses from a large randomized good clinical practice controlled trial with a stringent methodology and a high level of follow-up, there are limitations that deserve mentioning: First, follow-up for organ-related measures was not complete, although we followed patients for all blood samples done in 1) the hospital, at which they were initially recruited, 2) other hospitals in Denmark, where we had electronic access to blood samples. However, patients who continued to suffer from renal failure when discharged from hospital, were out of reach for follow-up for their renal function. Of note, the fraction of patients with remaining renal failure at time of discharge was comparable between the two groups (table 2), and hence it is unlikely that this lack of ability to ascertain renal outcome contributed to our main findings.

Second, eGFR may not be an accurate measure of creatinine clearance, as recently documented by Martin et al. [22]. However, even though this measure is not accurate to describe the creatinine clearance, changes in eGFR reflect changes in renal function, as validated, and is closely correlated to outcome[23]. Additionally, since hydration can be a source of error, we used the baseline weight in

the eGFR equation. Additionally, we found that eGFR<60 ml/min/1.73 m^2 on day 7 is a strong independent predictor of mortality.

Third, the RIFLE criteria used as secondary endpoint measures are not suitable to detect renal failure from baseline and forth, since the reference is defined as the pre-morbid creatinine. Hence, renal failure caused by exposure to antibiotics beginning at baseline, will not necessarily be captured using these criteria. This was the reason for using these as secondary endpoints.

Forth~~Third~~, the study was a post hoc analysis using a previously published trial as material. We have tried to compensate for this by writing a detailed analysis-plan based on the hypotheses, we wanted to test, before analysis. Fifth~~Third~~, although the sample size was relatively large compared to most other randomized trials in this setting, the sample size for these secondary analyses were based on the assumption of 25% renal failure in the 'standard exposure group' and a relative risk of 1.25 in the 'high exposure group'. The observed numbers were 21% and 1.22 which calls for a slightly higher sample size. However, the sample size needed to show the differences observed in the multivariable analyses was far smaller, and since these analyses confirmed the main findings, we do not think the results are due to chance.

In this trial, for the first time ever to our knowledge, random allocation to high exposure to broad-spectrum antibiotics in the intensive care unit has been systematically applied according to a systematic algorithm and this resulted in prolongation of renal failure. The results were confirmed when excluding patients with fungal infections, and a multiple effects model revealed a particularly low renal recovery in patients while piperacillin/tazobactam was administered and a remarkable recovery when discontinuing this drug; a finding that was specific for this drug. Several other crude and adjusted models likewise confirmed the findings. Finally, the results from this trial are supported by human experimental studies.

Conclusion

In conclusion, the use of piperacillin/tazobactam caused a delayed renal recovery in critically ill patients, and renal function improved after discontinuation of the drug. However, the study is not designed to investigate d*e novo* emergence of renal failure, since the lowest renal function is at baseline in most patients. ~~We cannot within the sample size and follow-up time of this trial~~The study was not designed to establish whether the use of piperacillin/tazobactam or other of the interventional drugs, in some cases cause~~s~~ persistent renal failure, and thus, further research to explore this is warranted. We think this impact on renal function is more likely caused by a – at least partially reversible - toxic effect on the renal tubule than by a lack of effect towards the infection, since this drug is independently associated with a high chance of survival in other infected populations[8], and we must emphasize that our findings are strictly confined to critically ill patients.

Contributors

JUJ designed the study, made the data collection tools, monitored data collection for the whole trial, wrote the statistical analysis plan, and drafted and the paper. He is guarantor. JUJ, ZF and JK cleaned and analysed the data. JL, BL, LH, MHB, TM, MHA, KJT, JL, MS, HT, PS-J, AØL, DGS, NR, KT, PCF, KML, NED, MEJ, LR, CØ, ZF, JK and JG made input study design, data collection tools and analysis plan and on the manuscript. JUJ implemented the trial at the centers. All members of the Procalcitonin And Survival Study (PASS) Group assisted in designing the trial. The members of the PASS study group are as follows: Central Coordinating Centre - J.U. Jensen, B. Lundgren, J. Grarup, M.L. Jakobsen, S. S. Reilev, M. Kofoed-Djursner, J. D. Lundgren; Regional Coordinating Centres - Hvidovre - J. Løken, M. Steensen; Gentofte - T. Mohr, K. Thornberg, K. Thormar; Hillerød - L.Hein, M. Bestle; Glostrup - D. Strange, A.Ø. Lauritsen; Herlev - H. Tousi, P. Søe-Jensen; Roskilde - N. Reiter, N.E. Drenck; Skejby - M.H. Andersen, P. Fjeldborg; Århus - K.M. Larsen; Data Management & Statistical Centre - Z. Fox, J. Kjær, D.

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(63) – K.M. Larsen, M. Goldinger, D. Illum, C. Jessen, A. Christiansen, A. Berg, T. Elkmann, J.A.K. Pedersen, M. Simonsen; Bispebjerg (14) H. Joensen, H. Alstrøm, C. Svane, A. Engquist. Supported by grants from the Danish Research Council, The Lundbeck Foundation, Research Foundation for the Capital Region of Denmark, The Toyota Foundation, Brahms diagnostica (un-restricted grant), The Harboe Foundation, The A.P. Møller Foundation and the Idella Foundation. None of these had any influence on the design or conduct of the study; collection, management, analysis, and interpretation of the data; nor the preparation, or approval of the manuscript. All authors had full access to all of the data in the study and conjointly take responsibility for the integrity of the data and the accuracy of the data analysis.

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Competing interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that the trial was funded mainly by the Danish State (Danish Research Council) and : all authors state that they have no relationships with companies that might have an interest in the submitted work in the previous 3 years; their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and all authors have no non-financial interests that may be relevant to the submitted work.

Ethical approval

The study was approved by the ethics committee for Copenhagen and Frederiksberg community (now Ethics Committee for the Capitol Region): H-KF-01-272-753. Patient consent: We received written consent from the patient or the next of kin for trial inclusion.

Data sharing

No additional data available.

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